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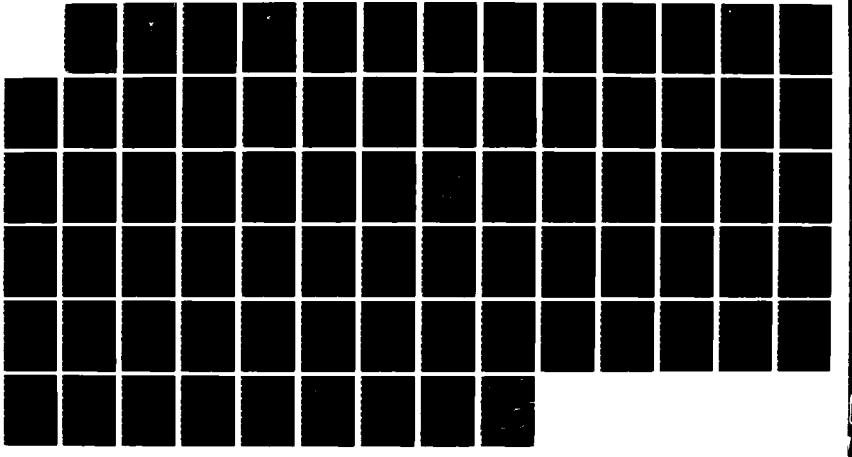

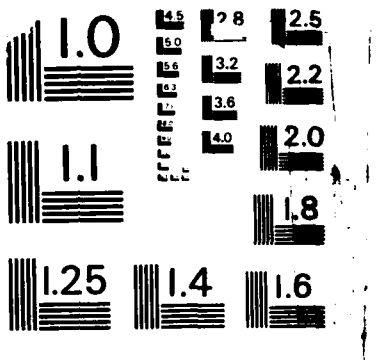

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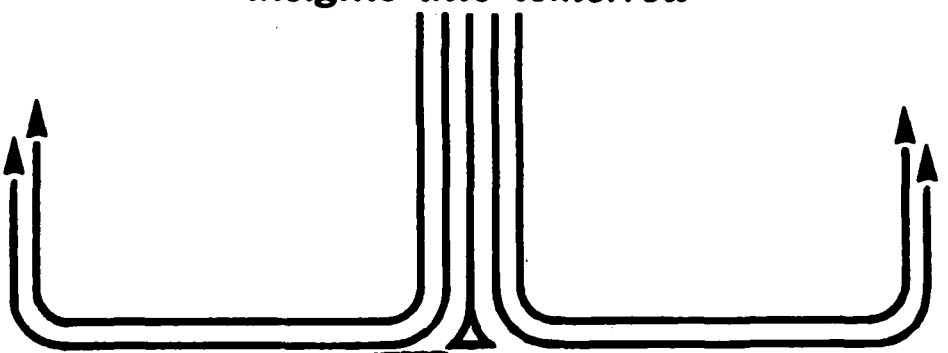
STUDENT REPORT

CORRELATION BETWEEN HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL) LEVEL AND AEROBIC ACTIVITY LEVEL

Major David S. Prewitt 87-2045
Major Kenneth A. Stafford

"insights into tomorrow"



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REPORT NUMBER 87-2045

TITLE CORRELATION BETWEEN HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL) LEVEL AND AEROBIC ACTIVITY LEVEL

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Submitted to the faculty in partial fulfillment of requirements for graduation.

AIR COMMAND AND STAFF COLLEGE
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The high density lipoprotein (HDL) fraction of total serum cholesterol has been identified as a cardiovascular disease risk-reducing factor. This technical study reports the correlation between aerobic activity level and measured HDL level among the subject population of mid-career US military officers. It concludes that a strong positive correlation exists between aerobic activity and HDL at low to moderate levels of exercise activity. This correlation becomes insignificant at high to very high levels of exercise activity. It also concludes that over a 12-week period, changes in aerobic activity level correlate positively with changes in HDL level.

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PREFACE

Cholesterol in the blood stream can be divided in several groups depending upon the relative density of the attached protein molecule. Recent studies have indicated that the high density lipoprotein cholesterol fraction can reduce the risk of cardiovascular disease. This study investigated the relationship of this good cholesterol to aerobic exercise.

The researchers are extremely grateful to the staff of the Maxwell Air Force Base Clinical Laboratory for their unhesitating support in this project. The members of the Air Command and Staff College Class of 1987 who enthusiastically participated in this study also deserve thanks for their willingness to fast, abstain from drinking, and survive two early morning blood drawings.

ABOUT THE AUTHORS

Major David S. Prewitt was born in Youngstown, Ohio on 10 January 1951. He attended North Georgia College from 1969 to 1973 where he received a Bachelor of Science degree in Physical Education. Upon graduation, he was commissioned a Second Lieutenant in the Regular Army in the Field Artillery Branch. Two years later, he completed the initial entry rotary wing course. Since then, he has completed aviation tours in Germany, Korea, and Ft. Rucker, Alabama. While at Ft. Rucker, he co-authored and received the monthly writing award from the Aviation Digest for his article "JAAT--A Present Concept." Major Prewitt completed a Masters of Science degree in Systems Management with the University of Southern California in 1982 and completed his second graduate degree in 1986 with a Masters in Physical Education from the University of Georgia. Following graduation from the Air Command and Staff College this June, he will be assigned as a member of the faculty in the Department of Physical Education, United States Military Academy, West Point.

Major Kenneth A. Stafford was born in Corvallis, Oregon on 4 June 1949. He attended Oregon State University from 1968 to 1972 where he first became interested in aerobic exercise through two Dr. Cooper-inspired physical education courses. Graduating with a Bachelor of Science degree in Mechanical Engineering, he went on to the USAF Officer Training School. Following his 1973 commissioning, Major Stafford attended Undergraduate Navigator Training and completed an initial flying tour in C-130 tactical airlift. In 1980, he graduated with distinction from the Air Force Institute of Technology residence program with a Master of Science degree in Aeronautical Engineering. His research there on vehicle design optimization and modelling was published and presented at the Institute of Electrical and Electronic Engineers Conference on Simulation. During his follow-on assignment as a contract management engineer, he also co-authored and published a monthly health awareness newsletter for installation-wide distribution. Returning to flying in 1984, Major Stafford spent a short tour in JC-130 aerial recovery operations before his present assignment at Air Command and Staff College. Following graduation in June 1987, he will be going on to fly with the HC-130 rescue squadron at RAF Woodbridge, UK.

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EXECUTIVE SUMMARY

Part of our College mission is distribution of the students' problem solving products to DoD sponsors and other interested agencies to enhance insight into contemporary, defense related issues. While the College has accepted this product as meeting academic requirements for graduation, the views and opinions expressed or implied are solely those of the author and should not be construed as carrying offic al sanction.

"insights into tomorrow"

REPORT NUMBER 87-2045

AUTHOR(S) MAJOR DAVID S. PREWITT, USA & MAJOR KENNETH A. STAFFORD, USAF

TITLE CORRELATION BETWEEN HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL) LEVEL AND AEROBIC ACTIVITY LEVEL

I. Purpose: To determine the correlation between high density lipoprotein cholesterol (HDL) level and aerobic activity level and to determine the degree that changes in aerobic activity level could affect the HDL level over a period of 12 weeks.

II. Problem: Cardiovascular disease remains the number one killer of American adult males. Recent research on prevention and/or reduction of this disease risk has established some 13 quantifiable cardiovascular risk factors. Several studies have now concluded that the high density lipoprotein (HDL) fraction of the serum cholesterol is one of the key factors in disease prediction. The same studies also highlighted the need for further investigation of the relationship between HDL and aerobic activity. This project is hence a technical report on such a study.

III. Procedures: Sixty-seven United States military male students of Air Command and Staff College (ACSC) Class 1987 were used as test subjects. The testing was accomplished in two phases. Data for the first test were obtained through blood testing conducted in late August/early September 1986 and completed activity surveys covering the July-August 1986 period.

CONTINUED

The Maxwell AFB Clinical Laboratory used the enzymatic Rapid Stat procedure to measure HDL levels. Aerobic activity levels were measured on a weekly basis using Dr. Kenneth Cooper's aerobic points methodology.

Approximately 12 weeks from the first test, a second blood test was conducted. Again the primary data of interest were individual HDL and aerobic activity levels. During both this phase and the initial testing, the subjects' behavior were also carefully monitored to prevent possible influences of diet, smoking, drinking, and medication from affecting test results.

With the data thus obtained, statistical analyses were initiated. The linear Pearson r coefficient of correlation was utilized to quantify the relationship between HDL and aerobic activity.

IV. Results and Discussion: Analysis of the data revealed some unexpected relationships. Data from the initial test phase showed fairly wide ranges of HDL for similar activity levels. Nonetheless, the computed HDL/aerobic points correlation was .58--more than twice as strong as needed to support the correlation hypothesis. With more stringent recording of activity data, the researchers expected an even more significant correlation coefficient for the second test data. In this case, however, the computed correlation was only .08--far too low to be significant. Curiously enough, even with this insignificantly related second test data, the 12-week change-effect hypothesis was supported. The correlation between changes in activity level with changes in HDL level over this period was a relatively strong .47.

In attempting to rationalize the above results, the researchers uncovered what was probably the most significant finding in the study. The average activity level of an individual had a profound effect upon the HDL/aerobic points correlation. After showing a high correlation at sedentary to moderate activity levels, this relationship dropped precipitously once high activity levels were obtained. In other words, the ideal subject would show remarkable increases in HDL as his activity level progressed from

CONTINUED

sedentary to 40 aerobic points per week. Further increases to 60 points per week would be accompanied with only slightly higher HDL. Between 60 and 160 points per week (the maximum tested), no further increases in HDL would be noted. Since the average activity level of the test subjects increased from 35 to 50 points during this study, this nonlinear correlation behavior did much to explain the second test's low computed correlation.

V. Conclusions: The correlation between HDL and aerobic activity is a nonlinear relationship. It is strongest at the lowest levels of aerobic activity, losing significance rapidly at moderate to high levels. At high levels of activity, the correlation approaches zero. Twelve weeks is sufficiently long to detect the effect of changes in aerobic activity on HDL level.

VI. Recommendations: Air University should continue to encourage the 30 aerobic-points-per-week Long Haul Program. Further research on this topic should concentrate on studying more low-to-moderate activity level subjects. Additionally, it should include reliability testing of the HDL measurement procedure and week-to-week variability testing of individual subjects.

Chapter I

INTRODUCTION

BACKGROUND OF THE STUDY

Approximately 55 percent of all deaths for 35- to 44-year-old American males is attributed to cardiovascular disease (5:3). The nature of the military's missions require its personnel to be in top physical and mental condition. Early deaths or incapacitations affect the readiness of the military, as well as increase its operating costs. A 1974 estimate of direct costs to the United States Air Force from cardiovascular death and disability exceeded $100 million annually (15:2). Clearly, effective means to assess and/or reduce the risk of cardiovascular disease are of significant interest to the military.

Researchers have now established some 13 primary risk factors that can be evaluated and combined to develop an individual's overall cardiovascular disease risk assessment (see appendix A). These factors can be broadly divided into controllable or uncontrollable. Those in the latter category include age, personal history, and family history. The concern of this study, however, was to investigate a component of one of the controllable factors, the so-called cholesterol ratio.

This ratio is the total serum cholesterol density divided by its high density lipoprotein (HDL) component. While considerable research has been documented on ways to control overall cholesterol levels, very little has yet been published on positive measures to independently affect the HDL proportion. Preliminary reports have indicated that HDL levels (and therefore the cholesterol ratio) may be affected by certain types of physical activity (10:149; 19:--). This study was developed to further test that assertion.

STATEMENT OF THE PROBLEM

This study was an investigation of the of the relationship between serum high density lipoprotein (HDL) level and aerobic exercise level.

PURPOSE OF THE STUDY

The primary purpose was to determine the correlation between HDL level and aerobic exercise level (as measured by weekly aerobic points). Additionally, it was to determine the degree that changes in aerobic activity level could affect the HDL level over a period of 12 weeks.

DELIMITATIONS OF THE STUDY

The study was delimited by the following:

1. The test group consisted of 67 United States military officers (all males), ranging in age from 32 to 43, enrolled in the Air Command and Staff College (ACSC) resident course during the 1986-1987 school year.

2. All correlations were determined using the Pearson product moment coefficient.

LIMITATIONS OF THE STUDY

The findings of this study were limited by the following factors:

1. The generalizability was limited to male United States military officers ages 32 to 43.

2. Each subject's blood was analyzed only once at the beginning and then once again at the end of the 12-week period; therefore, test reliabilities were not shown through the data collection.

3. The first blood test was conducted prior to the formal initiation of this study. This prevented the researchers from pre-briefing the subjects on the activity data required for the first survey.

4. The number of subjects was limited to a maximum of 80 due to the clinical laboratory workload at Maxwell AFB.

HYPOTHESES

The hypotheses tested in this study were as follows:

1. A significant positive correlation between HDL level and aerobic level exists at the .05 level of probability.

2. A significant positive correlation between changes

in HDL level and changes in aerobic level over a 12-week period exists at the .05 level of probability.

BASIC ASSUMPTIONS

The following assumptions were made concerning this study:

1. All subjects reported their aerobic activity level accurately in accordance with the researchers' instructions.

2. The lab technicians provided the researchers with reliable data.

3. Dr. Cooper's method of determining aerobic points is consistent for each activity that was used by the subjects (6:172-185).

4. Each subject fasted 12 to 14 hours and refrained from alcohol consumption 48 hours before each blood test as requested.

5. The physical capacities of the subjects were typical of United States military males ages 32 to 43.

6. No significant, undocumented dietary changes were made by the subjects during the test period.

7. Environmental factors outside the scrutiny of this study had a homogeneous effect on the subject group.

DEFINITION OF TERMS

Interpretations of the following terms for this study were:

1. Aerobic exercise--"Those activities that require oxygen for prolonged periods and place such demands on the body that it is required to improve its capability to handle oxygen" (4:13).

2. Aerobic fitness--The relative measure of an individual's maximum capacity to utilize oxygen during prolonged exercise. Fitness categories ranging from very poor to excellent (levels I to V) can be assigned based upon a 1.5 mile run test (6:31).

3. Aerobic points--An aerobic exercise accounting methodology established by Dr. Cooper (3:95). They are essentially assigned to exercises on a basis of required

oxygen usage rate per unit body mass times exercise duration.

4. Cardiovascular disease--Any of a variety of diseases resulting from a build-up of fatty cholesterol deposits in the blood vessels (hypercholesterolemia) which leads to clogging of arteries (atherosclerosis) and eventual loss of elasticity of the coronary and other arteries (arteriosclerosis).

5. Cardiovascular risk factor--Any of a number of factors identified by the American Heart Association (and others) that have been associated with the prediction and/or development of cardiovascular disease.

6. Coefficient of correlation (Pearson r)--The value of the linear relationship between two variables. This coefficient can range from 0.0 to +/- 1.0. No relationship is indicated by 0.0, while a +1.0 and -1.0 represent a perfect positive and negative correlation respectively. A lower case r (e.g. r = +.5) is used to represent the Pearson correlation coefficient.

7. High density lipoprotein (HDL)--The smallest and highest density fat-carrying proteins in the blood. The presence of HDLs has been hypothesized as negating the damaging effects of low-density lipoprotein (LDL) and is often called the "good guy."

8. Level of probability--The stated level at which the relationship could have been attributable to chance occurrence (for example, at the .05 level of probability, there exists a 5 percent possibility that a determined correlation occurred by chance).

Chapter II

REVIEW OF RELATED LITERATURE

The primary purpose of the study was to determine the correlation between HDL levels and aerobic exercise levels (as measured by weekly aerobic points). Additionally, it was to determine the degree that changes in aerobic level could affect the HDL level over a period of 12 weeks.

The basics of the cardiovascular system were described as early as 1628 in William Harvey's thesis "On the Movement of the Heart and Blood in Animals" (1:ix). However, it has only been in the last 30-40 years that researchers have attempted to connect certain behaviorial and inherited traits to cardiovascular disease and coronary mortality. Additionally, only within the last decade have studies suggested the preponderate effect of serum cholesterol ratio on the overall disease inclination. Positive means of controlling this potentially critical factor have only just recently been researched.

The review of literature related to this study parallels the research sequence stated above and is divided into three major sections. The first section, cardiovascular risk factors, addresses the basic formulation of cardiovascular risk factors that establishes overall risk assessment. The next section, cholesterol ratio, highlights specific material concerning the cholesterol ratio risk factor. The last section, HDL and aerobic exercise, reviews the latest literature relating cholesterol ratio (specifically HDL) to aerobic exercise.

CARDIOVASCULAR RISK FACTORS

The pioneering study on the development and prediction of cardiovascular disease was clearly the Framingham Study (1:ix). Beginning in 1948, the study has followed the lives of 5200 men and women from Framingham, Massachusetts. After some 30 years of observation, researchers established five major characteristics associated with increased incidence of cardiovascular disease and/or mortality. These initial predictors or "risk factors" were 1) high blood pressure

(hypertension), 2) elevated serum cholesterol level, 3) elevated blood sugar, 4) cigarette smoking, and 5) obesity (overeating with too little exercise).

Concurrent to the Framingham Study and also a landmark report was the Seven Countries Study conducted in the 1950's and 1960's (1:3). Eighteen communities in Europe, Asia, and the United States, initially involving 12,000 men, were studied. A significantly high correlation between serum cholesterol and cardiovascular disease was again established.

Several other major studies have contributed to these initial projects, resulting in the American Heart Association presently endorsing 13 significant risk factors. These factors, listed in approximate descending order of importance are 1) cholesterol ratio, 2) serum cholesterol, 3) smoking, 4) personal history (of coronary events), 5) cardiovascular fitness, 6) psychological stress, 7) age factor, 8) diastolic blood pressure, 9) systolic blood pressure, 10) family coronary history, 11) body composition (percent fat), 12) fasting blood sugar, and 13) serum triglycerides (16:1-41). Note that while the initial five factors identified by the Framingham Study are included, a new risk factor, cholesterol ratio, has now been identified as the most significant.

CHOLESTEROL RATIO

The existence of at least two types of serum cholesterol, HDL and low density lipoprotein (LDL), has been recognized for many years. Only since 1975, however, have researchers correlated elevated levels of HDL with low cardiovascular risk (9:708; 14:79). Thus, with the already recognized association of total cholesterol with cardiovascular disease, a new risk factor was proposed. This factor was the total serum cholesterol level (easily measured) divided by its HDL component level (requiring much more rigorous laboratory work) (4:85).

The recent studies concerning the cholesterol ratio, point toward the ratio being more critical than the combined total cholesterol level (11:85). Based upon ongoing research at his Aerobics Center, Dr. Cooper stated in 1982 that this ratio ". . . is perhaps the single most important factor in predicting your susceptibility to heart attacks, and in determining your total well-being, both now and in the future" (4:85). His research has indicated the following:

1) Very low total cholesterol levels do not necessarily reduce risk when associated with high ratios (i.e. low HDL level). In one instance, a subject with an unusually low total cholesterol level (147 mg/dl compared to the commonly

suggested safe level of 200) nevertheless experienced a massive myocardial infarction (heart attack) indicating advanced atherosclerosis. His measured HDL level, however, was only 22. This computed to a ratio of 6.7--far higher than the suggested safe value of 4.5 or less (4:86).

2) On the other hand, high HDL level, while seemingly associated with longevity, does not in itself eliminate coronary risk when combined with high total cholesterol. One of Dr. Cooper's subjects required extensive surgery to bypass several severely clogged coronary arteries even though his HDL level of 60 was well above average. Again, his ratio was nearly 7.0 due to his total cholesterol level of almost 400 (4:86).

3) The influence of this ratio may well be the determining factor why women generally have a lower incidence of cardiovascular disease than men. Total average cholesterol levels show no significant sexual bias, however women, especially with increasing age, tend to have higher HDL levels. Above the age of 60, Dr. Cooper reported a resulting average cholesterol ratio of 4.8 for men compared with a much more favorable 3.6 average for women (4:87).

Several other minor studies have supported Dr. Cooper's findings. However, the major Lipid Research Clinic's Coronary Primary Prevention Trial (LRC-CPPT) completed in 1984 had an even more significant conclusion. This $150 million, 12-year study not only demonstrated the recognized correlation between both elevated total cholesterol and low ratios with increased risk, but for the first time, showed positively that these were indeed causal factors. In this closely controlled study of 3806 adult males with 193,000 clinic visits, two carefully matched groups were established. While one group was given a placebo, the other was given an LDL-reducing/HDL-increasing drug (cholestyramine). All other risk factors were monitored and remained matched throughout the study. In the end, the group that had lowered their cholesterol ratio had a significantly lower incidence of fatal and nonfatal cardiovascular diseases (12:41; 14:76, 79-80).

The conclusions from the most recent studies clearly motivate further research on measures to improve/lower the ratio. Several well-documented methods exist for reducing LDL which has the net effect desired (4:91). These include a low-cholesterol diet, weight reduction, and certain medications. On the other side of the equation, new studies are beginning to focus on a possible causal relationship between HDL level and aerobic level.

HDL AND AEROBIC EXERCISE

Without attempting to establish the physiological mechanism, several researchers have stated that HDL appears to increase with sustained aerobic training programs. Two recent Stanford studies have noted that both male and female long-distance runners had more HDL than their analogous nonrunning control groups (5:34). The multi-million dollar LRC-CPPT project also concluded that endurance exercises such as running, brisk walking, cycling, and cross-country skiing led to increased HDL (14:80). At Dr. Cooper's Aerobics Center, analysis has shown that not only does aerobic exercise of any type lead to increased HDL, but overall measured cardiovascular fitness positively correlates with HDL (4:88).

While studies beginning with the historical Framingham Study have linked active life styles with reduced coronary risk, only the most recent researchers have suggested that the effect may largely be due to changes in HDL level. Currently, there are no published reports comprehensively analysing this highly probable relationship, even though the National Institute of Health recorded in a 1980 Consensus Development Conference Statement that "more information is needed with regard to factors controlling the level of HDL . . . " (13:10).

Chapter III

PROCEDURES

The primary purpose of the study was to determine the correlation between HDL level and aerobic exercise level (as measured by weekly aerobic points). Additionally, it was to determine the degree that changes in aerobic level could affect the HDL level over a period of 12 weeks. The methods (procedures) used to collect and analyze the data are described in this chapter. The chapter includes a description of the subjects, selected test parameters, data collection, equipment, and data analysis.

SUBJECTS

The subjects were United States military male students in the 1986-1987 class of Air Command and Staff College (ACSC) at Maxwell AFB, Alabama. They were mid-career officers (primarily U. S. Air Force), and aged 32 to 43, selected for ACSC attendence by virtue of their exemplary military records and potential for promotion. Otherwise, they closely represent a cross section of their military peers with regard to career fields, physical stature, health, and fitness. As with all military personnel, they were required to maintain their body weight below certain maximum standards and maintain a minimum cardiovascular fitness level. This fitness standard (measured periodically by an aerobic run test) equates closely to level III ("fair") on Dr. Cooper's fitness scale (6:31).

Only the 341 such students who volunteered to have their blood tested for HDL level and other cardiovascular risk factors at the beginning of the academic year were considered for this study. Due to laboratory analysis limitations, 100 of these 341 were subsequently selected by computer simple randomization for possible participation in the further testing required. Nevertheless, the researchers were reasonably confident that subjects would fall into all categories of activity levels. Additionally, it was expected that these subjects would have changes in activity levels ranging from decreased to increased (including no change). Of these 100, 88 completed the initial activity

survey/questionaire. Sixty-seven of these 88 eventually submitted to the second blood test and completed the second survey, which included a detailed record of their aerobic points during the approximate 12 weeks between blood tests. This eventual sample size technically represents the subject group to a 91 percent/plus-or-minus nine percent confidence/precision level. Furthermore, as can be seen in the following table (Table 1), this sample very closely represented the subject group on selected parameters obtained during the first blood test.

Selected Parameters	Sample (67)	Subject Group (341)
Age (years)/SD	35.8/2.3	36.1/2.9
Height (inches)/SD	70.5/2.3	70.1/3.0
Weight (pounds)/SD	173.7/20.6	170.4/22.4
Total Cholesterol (mg/dl)/SD	189.0/31.9	186.4/34.9
HDL (mg/dl)/SD	46.7/9.9	46.5/10.1

Table 1. Mean Selected Initial Blood Test Parameters with Standard Deviation (SD)

TEST PARAMETERS

The two parameters of primary concern for this study were the aerobic level and the HDL levels for each subject. Aerobic level was tracked via an aerobic point system while HDL levels were obtained via an HDL blood test.

Aerobic Point System--Aerobic points were determined using the method described in Dr. Cooper's The New Aerobics (6:172-185). Subjects were asked (as part of the first survey) to recall their average weekly aerobic level for the period 1 July to 31 August 1986 (the period just prior to the first blood test). To aid in this determination, charts were designed using Dr. Cooper's method as the basis (see Appendix B).

Subjects were then directed to keep an accurate record of their aerobic points for the period 29 September through 23 November 1986. (As a requirement of the ACSC curriculum, all officers were required to obtain a minimum of 30 aerobic points per week--the ACSC Long Haul program.)

HDL Blood Test--Blood samples were collected and tested for HDL level twice during this study. The first samples

were collected during the period 25 August through 1 September 1986. The later samples were drawn approximately 12 weeks later on 24 November 1986. In both cases, the blood-taking, sample-handling, and laboratory testing were accomplished by trained laboratory personnel from the Maxwell AFB Clinical Laboratory. The actual sample collections were accomplished at four stations set up next to the ACSC Snack Bar. Both collections were taken in the morning hours between 0630 and 0815 following a 12- to 14-hour subject fasting period.

Self-adhesive lab slips for the sample containers were prepared in advance by the researchers to expedite processing and insure standardization and positive identification of all blood samples.

The Lancer Rapid Stat procedure was used to determine the fasting serum HDL level for each subject. This procedure utilized both the cholesterol oxidase and cholesterol esterase in conjunction with the peroxidase/phenol/4-aminoantipyrine system outlined by Trinder (1969). The precipitant reagent used was phosphotingstic acid. For a thorough explanation of the procedures used, see Appendix C.

DATA COLLECTION

Data was collected for this study via health assessment worksheets, lab sheets, and activity surveys.

Health Assessment Worksheet--This worksheet was initially designed for a separate health risk factor study and, as such, contained several items of only secondary interest to this study. It was filled out by each participant with the aid of the laboratory technician just prior to the first blood test. Information recorded included name, student number, seminar number, age, height, weight, systolic blood pressure, diastolic blood pressure, resting heart rate, and the use of any medication (see Appendix D).

Lab Sheet--Laboratory analysis results were transcribed onto individual lab sheets by the laboratory personnel at Maxwell AFB for both blood tests. The researchers used the annotated total cholesterol and HDL levels to calculate the cholesterol ratio. After marking this ratio on the lab sheets, individual copies were distributed back to the subjects. The completed sheets contained the following information: name, sex, age, glucose level, cholesterol level, triglyceride level, HDL level, and the cholesterol ratio (see Appendix E).

Survey--Two activity surveys were constructed.

Activities/behaviors surveyed on both forms included alcohol consumption, use of cigarettes, performance on last aerobic fitness test (1.5 mile run), frequency of aerobic exercise sessions, and weekly aerobic exercise points. The first survey was distributed to the 100 potential research subjects following the first blood test. A cover letter explaining the study was attached. The researchers also conducted a mass briefing for the sample group, highlighting the need for accuracy in completing the form, going over the questions in detail, and especially, reviewing the unique aerobic point activity charts which were included with each survey form. A final question on this first survey queried whether the subject was interested in continuing the study by closely recording aerobic level and later submitting to a second blood test (see Appendix B).

The second survey was similar to the first. It was handed out to those subjects desiring to remain in the study. Distributed just prior to the second blood test, this form was collected and quality-checked during the sample collection. Age, weight, and height were recorded as well as the week-by-week aerobic levels since the first blood test (see Appendix F).

EQUIPMENT

Equipment for this study consisted of the following:

1. Sliding-weight beam balance, capable of measuring to the nearest 1/2 pound of weight and 1/2 inch of height.

2. Blood sample containers.

3. Boehringer Mannheim Diagnostic enzymatic cholesterol test kit 625220, for total cholesterol test.

4. Lancer Rapid Stat diagnostic kit, for HDL test.

5. Boehringer Mannheim Diagnostics model 8700 spectrophotometer, for measuring cholesterol and HDL levels.

DATA ANALYSIS

All data were entered onto a data base using LOTUS 1-2-3 software on a Tandy 1000 personal computer. The data included that obtained via the previously described health assessment worksheets, lab sheets, and activity surveys, as well as several derived parameters. These included the cholesterol ratio (for both sets of test data), a points change factor (aerobic points recorded on the first survey

subtracted from that on the second survey), and an HDL level change factor (HDL from the first blood test subtracted from that on the second test).

Statistical analysis (means, standard deviations, and Pearson correlations) of the data collected during both test phases was accomplished via an EPISTAT 3.1 statistical software package. Additionally, the Pearson correlation was calculated between the HDL level change factor and points change factor. The significance of these correlations at the .05 level of probability was used to accept or reject the hypotheses. Percentile ranks for aerobic fitness points were manually determined for the subjects.

Chapter IV

RESULTS AND DISCUSSION

The primary purpose of the study was to determine the correlation between HDL levels and aerobic exercise level (as measured by weekly aerobic points). Additionally, it was to determine the degree that changes in aerobic level could affect the HDL level over a period of 12 weeks. The researchers hypothesized that (a) there was a significant positive correlation between HDL level and aerobic level at the .05 level of probability, and (b) there was a significant positive correlation between changes in HDL level and changes in aerobic level over a 12-week period at the .05 level of probability. For testing the hypotheses, the Pearson correlation coefficient between the scores from the two tests administered to each subject was used. Percentile ranks for the aerobic activity level were also computed.

The presentation of the findings and the discussion of the data analysis are divided into four sections. The sections are descriptive statistics, percentile rank, simple correlation, and discussion of results. Throughout this chapter, all measurements related to HDL or cholesterol levels are in units of mg/dl and all aerobic points recorded are 12-week weekly averages. Additionally, alcohol usage is measured in one-ounce hard liquor equivalents per week, cigarette smoking in cigarettes per day, fitness in the categories established by Dr. Cooper (6:31), and sessions in the number of aerobic exercise periods per week.

DESCRIPTIVE STATISTICS

The scores (risk factors) from single samples were used as the criterion for each of the items associated with the blood analysis (cholesterol, triglyceride, HDL level, and HDL ratio). The following scores were also used, and were obtained from a single survey given after the initial blood samples were drawn. These were alcohol consumption, cigarette usage, last measured fitness level (via 1.5 mile run), average exercise session frequency, medication used, and the number of weekly aerobic points earned during the last two months. The same information was obtained 12 weeks

after the first blood test. However, this time the subjects had been directed to keep a weekly log of their aerobic activity. The mean, standard deviation, and range of scores for the above items are displayed in Tables 2 and 3.

PARAMETER	MEAN	SD	RANGE
CHOLESTEROL	189.0	31.9	121-263
TRIGLYCERIDE	98.7	42.4	42-237
HDL	46.7	9.9	32-78
RATIO	4.3	1.3	2.2-8.2
ALCOHOL	3.5	4.2	0-15
CIGARETTE	1.1	4.6	0-25
FITNESS LEVEL	4.2	.8	2-5
# SESSIONS	2.6	1.7	0-6
AEROBIC PTS	34.9	28.8	0-160

Table 2. Mean Parameters, Standard Deviation (SD), and Range (sample # 1 & survey # 1).

PARAMETER	MEAN	SD	RANGE
CHOLESTEROL	188.4	31.0	129-259
TRIGLYCERIDE	104.3	39.2	49-223
HDL	45.7	7.0	36-70
RATIO	4.2	0.8	2.7-7.0
ALCOHOL	3.8	4.2	0-15
CIGARETTE	0.9	4.0	0-25
FITNESS LEVEL	4.2	0.7	3-5
# SESSIONS	3.9	1.3	0-6
AEROBIC PTS	49.7	28.5	11-162

Table 3. Mean Parameter, Standard Deviation (SD), and Range (sample # 2 & survey # 2).

PERCENTILE RANK (PR)

Presented in Tables 4 and 5 are the percentile ranks for the subjects' aerobic activity level at the beginning and the end of the study respectively. A feature of note in these tables is the percentile rank corresponding to 30 aerobic

PTS	# SUBJECTS	PR
160	1	99
120	1	98
106	1	96
85	1	95
66	3	92
60	2	88
58	2	85
52	1	83
50	3	80
47	2	76
45	3	72
44	1	69
43	1	68
40	2	66
39	3	62
37	1	59
34	3	56
33	2	52
32	2	49
30	5	44
29	1	40
26	1	38
25	3	35
24	1	32
21	1	31
20	1	29
14	1	28
13	1	26
12	2	24
11	1	22
10	2	19
9	1	17
7	1	16
5	1	14
3	1	13
0	8	6

Mean 35
SD 29

Table 4. PR for Activity Levels at Beginning of Study

PTS	# SUBJECTS	PR
162	1	99
130	1	98
120	1	96
110	1	95
108	1	93
104	1	92
88	1	90
86	1	89
80	1	87
78	1	86
71	1	84
68	1	83
68	1	81
63	2	79
62	1	77
59	1	75
58	1	74
57	1	72
56	2	70
53	2	67
50	1	65
48	1	63
47	2	61
46	1	59
44	3	56
42	1	53
39	2	51
37	1	49
36	4	45
35	2	40
34	2	37
33	2	34
32	6	28
30	8	18
29	2	10
28	1	8
26	1	7
19	2	4
18	1	2
11	1	1

Mean 50
SD 29

Table 5. PR for Activity Levels at End of Study

points. Dr. Cooper found that 80 percent of the persons who had obtained approximately 30 points per week would reach his minimum level of fitness (6:19). The percentile norms in Table 4 had a greater percentage of subjects falling below the 30-point level than the percentile norms in Table 5. This would indicate that a greater percentage of subjects were likely to be fit toward the end of the study.

SIMPLE CORRELATION

The correlation matrices for all test parameters for both sets of data are presented in Appendix G. The simple correlations, Pearson r, between HDL level and activity level, and between changes in HDL level and changes in aerobic level over a 12-week period, were determined to test the hypotheses of this study. The hypotheses warranted the use of a two-tailed test at the .05 level of probability to determine acceptance or rejection.

The correlation was .58 between HDL level and activity level for the first set of data, and .08 between HDL level and activity for the second set of data. The hypothesis was accepted for the first set of data, as $r = .58$ was significantly different from zero at the .05 level of probability ($r = .58$ is actually significant at the much more stringent .001 level of probability). The hypothesis was rejected for the second set of data, as $r = .08$ was not significantly different from zero at the .05 level of probability.

The correlation was .47 between changes in HDL level and changes in aerobic level over the 12-week period. The hypothesis was accepted, as $r = .47$ was significantly different from zero at the .05 level of probability.

DISCUSSION OF RESULTS

In analysing the aggregate data, in view especially of the discrepant correlations between HDL and aerobic points mentioned above, several approaches were taken.

Analysis by Activity Changes

As was anticipated by the researchers, the subjects could be divided into three groups (A, B, and C) based upon changes in their average weekly aerobic points between the two data collection phases. All subjects who increased their activity levels by more than 10 percent were included in Group A. Group B included those who reported a more than 10 percent decrease in activity. Finally, those who did not

significantly change their activity level were placed in Group C. This last Group was considered a control group. The mean change in Group C's HDL level (-7.3) occurred in the absence of aerobic activity level changes and was therefore assigned to unknown environmental and/or laboratory calibration factors. From whatever source (ACSC stress factors, seasonal factors, laboratory techniques, etc.), this influence was assumed evenly distributed among the subjects and, therefore, was used to normalize the second HDL test scores to the first.

Displayed in Table 6 are the groups' aerobic points and HDL data. After applying the +7.3 normalizing correction, an HDL/points sensitivity factor was also derived. This factor is the group's mean change in HDL divided by their mean change in aerobic points. A final factor included in Table 6 is the average mean points which represents the simple average of each group's mean points during each test phase. This factor was used in later analysis.

CHARACTERISTIC	GROUP A (increased)	GROUP B (decreased)	GROUP C (no change)
NO. OF SUBJECTS	41	9	17
MEAN PTS (#1)	22.8	49.6	56.5
MEAN PTS (#2)	50.0	38.3	55.6
CHANGE IN PTS	+27.2	-11.7	~0
MEAN HDL (#1)	43.0	55.6	50.8
MEAN HDL (#2)	46.8	44.9	43.5
CHANGE IN HDL	+ 3.8	-10.7	- 7.3
(NORMALIZED)	+11.1	- 3.4	0.0
SENSITIVITY	+ .41	+ .29	N/A
AVG MEAN PTS	36.4	44.0	56.1

Table 6. Analysis by Activity Changes

The prime purpose of this analysis approach was to obtain a factor to normalize the HDL data from the second test to the first. This +7.3 factor was indeed used throughout the remainder of this study. An interesting added relationship, however, also apparent in Table 6, was that of the average mean points to the HDL/points sensitivity (the relative influence of changes in points to changes in HDL). A nonlinear (i.e. changing slope or sensitivity) relationship was implied. Further approaches were used to investigate this relationship in more detail.

Analysis by Initial Activity Level

To more fully understand the relationship between a subject's nominal activity level (average mean points) and HDL/points sensitivity, a second grouping of data was evaluated. For this analysis, the subjects were divided into four groups (D through G) based upon their initial reported average activity levels. The results of this approach by initial activity level are displayed in Table 7.

CHARACTERISTICS (initial pts level)	GROUP D 0-10	GROUP E 11-30	GROUP F 31-55	GROUP G 56+
NO. OF SUBJECTS	14	18	24	11
MEAN PTS (#1)	3.4	22.6	41.0	82.3
MEAN PTS (#2)	30.9	47.5	46.3	86.9
CHANGE IN PTS	+27.5	+24.9	+ 5.3	+ 4.6
MEAN HDL (#1)	37.9	43.3	51.1	53.5
MEAN HDL (#2)	48.2	47.3	42.8	46.2
CHANGE IN HDL	+10.3	+ 4.0	- 8.3	- 7.3
(NORMALIZED)	+17.6	+11.3	- 1.0	0.0
SENSITIVITY	+ .64	+ .45	- .19	.00
AVG MEAN PTS	17.2	35.0	43.7	84.6

Table 7. Analysis by Initial Activity Level

Again, a nonlinear relationship appeared. The highest sensitivity was associated with the lowest average activity levels. Those subjects who began with almost no aerobic training program had clearly the largest increase in HDL per increased increment of activity. In fact, while considerable data spread existed throughout the subjects, all 14 subjects in Group D experienced an increase in HDL level with their increases in activity level. Groups F and G had very small net changes in points between the two tests which led to increased uncertainty in their sensitivity calculations.

Analysis by Average Mean Points

To establish a meaningful overall relationship between HDL level/points sensitivity and basic aerobic activity level, an additional data point was required to augment the high uncertainty sensitivities displayed in Groups F and G in Table 7. A final analysis group was therefore established.

Subjects for this Group H were selected as those who had

significant activity level changes (greater than 10 percent between tests), whose initial points were greater than 30, and whose two-test average points were greater than 50. Table 8 displays this data.

CHARACTERISTICS	GROUP H
NO. OF SUBJECTS	12
MEAN PTS (#1)	50.7
MEAN PTS (#2)	66.7
CHANGE IN PTS	+16.0
MEAN HDL (#1)	51.0
MEAN HDL (#2)	45.0
CHANGE IN HDL	- 6.0
(NORMALIZED)	+ 1.3
SENSITIVITY	+ .08
AVG MEAN PTS	58.7

Table 8. Additional Analysis Group

Shown in Table 9 is the composite listing of the derived sensitivites versus average mean points obtained for Groups A through H.

AVG MEAN PTS	SENSITIVITY	GROUP
17.2	+.64	D
35.0	+.45	E
36.4	+.41	A
43.7	-.19	F
44.0	+.29	B
56.1	N/A	C
58.7	+.08	H
84.6	+.00	G

Table 9. HDL/PTS Sensitivity Versus Average Mean Points

Clearly, Table 9 data revealed an inverse relationship between the subject groups' average aerobic activity levels and their HDL level sensitivity to further changes in

activity level. Due to the relatively small changes in activity level by Groups F and G, the uncertainty in their respective sensitivities was much larger than that of the other groups (see Appendix H). Since Group C was the control group with no significant activity changes, a sensitivity calculation was not appropriate. Figure 1 graphically shows the Table 9 data. With the exception of Group F's data, the calculated sensitivities show a remarkably smooth relationship.

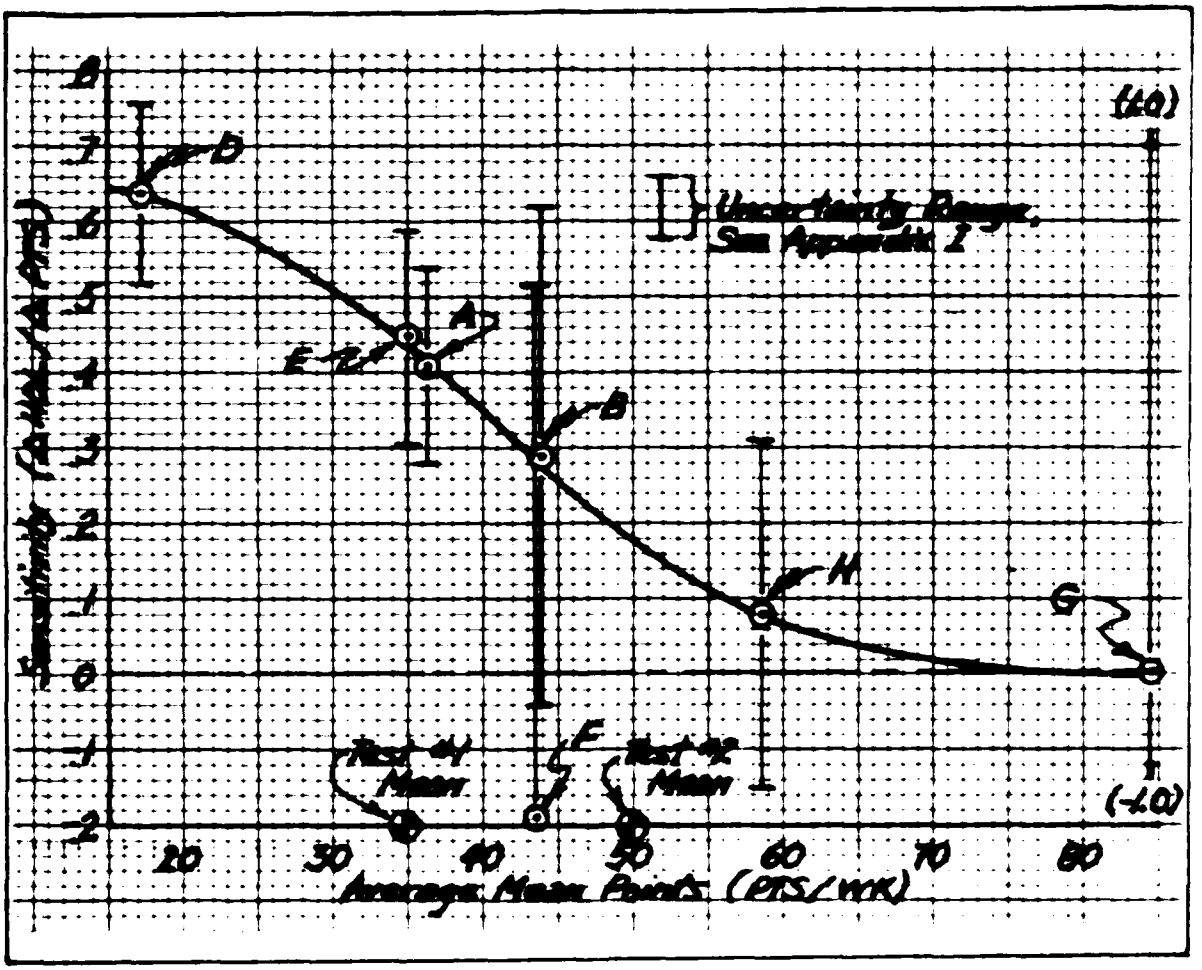

Figure 1. HDL/PTS Sensitivity Versus Average Mean Points

From the data depicted in Figure 1, there appeared to be a strong reduction in HDL level sensitivity to aerobic exercise once a moderate level of exercise was achieved (about 30-40 points per week). In fact, at high levels (greater than 60 points per week) the slope of the curve approached zero.

Specific Analysis of First and Second Test Correlations

The relationships shown in Figure 1 affected the calculated HDL/points correlations in a significant manner. First, the nonconstant sensitivity indicated a nonlinear relationship between HDL and aerobic points. This fact, which can also be seen in the raw data (shown in Appendix I) automatically leads to an artifically low estimation of correlation when using a linear algorithm such as the Pearson coefficient (2:75).

With relatively constant standard deviations for test parameters, the Pearson coefficient is proportional to the slope of the regression equation (2:75). This slope equates to the HDL/points sensitivity referred to above. It follows that a reduction in slope (or sensitivity) directly results in a lower computed correlation. Therefore, a second effect of the relationship in Figure 1 was a predictable lowering in correlation between the first and second tests. The mean points level increased from 35 to 50 which led to a mean sensitivity drop from .45 to .18. Also, more than twice as many subjects in the second test were above the 60-points level (see Appendix I) where the predicted sensitivity (re correlation) approached zero.

These combined effects of the sensitivity/points relationship do much to explain the gross distribution of the test data.

Analysis of Case-by-Case Anomalies

The raw data (displayed in Appendix I) contained considerable scatter. Particularly noteworthy were the large changes (+/- 20) in HDL recorded for several of the subjects A careful case-by-case review of the respective surveys, lab sheets, health assessment worksheets, and, in some cases, personal interviews, failed to produce a significant justification for those radical changes. Neither could discrepancies be determined in reviewing the laboratory quality control data. For these reasons, no data was removed from consideration in the correlation studies.

An additional study of the limited number of subjects who either changed their dietary habits (n=1), alcohol consumption (n=13), or use of cigarettes (n=2), or for those subjects under any type of medication within the preceding 30 days of each blood test (n=4), failed to demonstrate any significant effect on HDL levels.

Interpreting Meaningfulness

Interpreting a correlation coefficient as to its meaningfulness is commonly done using the coefficient of determination (the square of r). Using this method, the portion of common association of the factors which influences the two variables can be determined (8:105-107). The coefficient of determination for .58 and .08 is .34 and .01 respectively. Expressing these figures in terms of variation results in 34 percent and 1 percent. This is to say that in the first case, a little over a third of the variance (or influences) in the HDL levels was associated with the variance in the aerobic activity levels. However, in the later test, as the activity level increased, there was virtually no variance or influence for the HDL levels and the aerobic activity levels.

Chapter V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The primary purpose of the study was to determine the correlation between HDL level and aerobic exercise level (as measured by weekly aerobic points). Additionally, it was to determine the degree that changes in aerobic activity could affect the HDL level over a period of 12 weeks. The contents of this chapter are presented under the following major headings: summary, conclusions, and recommendations.

SUMMARY

One hundred subjects were randomly selected from a group of 341 United States military male officers attending the Air Command and Staff College at Maxwell AFB, Alabama. These 341 subjects were volunteers in a separate blood test study conducted in August/September 1986 to determine their cardiovascular risk factors. Of the randomly sampled 100, 67 participated fully in this follow-on study. Data from the original blood test as well as a later second test were evaluated. Aerobic level data in the form of aerobic points were obtained from two surveys covering the subjects' behavior in the 10- to 12-week period prior to each blood test.

The aggregate data from the surveys and the blood samples were used by the researchers for analysis. The Pearson correlation between aerobic activity level and HDL level was calculated and examined for the two time periods. Additionally, the Pearson correlation between changes in aerobic level and changes in the HDL level over the 12-week study period was calculated and examined. Percentile rank norms were also calculated for the aerobic activity levels of the subjects for the two time periods.

Once the above computations were made, the researchers examined the various groupings of data. The percentile rank norms showed a larger percentage of subjects meeting Dr. Cooper's minimum fitness standards at the end of the study than at the beginning. Further analyses of the groups revealed correlations of .58 (significant at the .001 level of

probability) and .08 (which was not significant at the .05 level of probability) between aerobic activity level and HDL level for the beginning and the end of the study respectively. There was also a .47 correlation (significant at the .001 level of probability) between changes in aerobic activity levels and attendant changes in the HDL levels over the study period of 12 weeks. These correlations resulted in the researchers' first hypothesis being accepted for certain conditions and being rejected under other conditions. The researchers' second hypothesis was accepted.

The significant positive correlation between aerobic activity levels and HDL levels determined for the initial data supported the researchers' hypothesis and was in accord with other researchers' implications. However, the low correlation for the later data prompted an analysis which revealed a nonlinear relationship between HDL level and aerobic activity levels. At aerobic activity levels above 30 aerobic points per week, the apparent sensitivity of HDL level to activity level changes dropped sharply. At 60 or more aerobic points per week, further increases in activity were, in fact, not associated with further changes in HDL level. The difference between the two HDL level/aerobic activity level correlations determined in this study was largely explained by the gross increase in average subject activity level from the beginning to the end of the study.

The significant correlations determined in this study suggest that lifestyle changes that include going from a sedentary to moderate activity level are associated with increases in HDL level. This, in turn, has a favorable effect on the ratio of overall cholesterol to HDL, which is recognized as the most significant individual cardiovascular disease risk factor.

CONCLUSIONS

The following conclusions were supported by the findings of this study:

1. Aerobic activity levels and HDL levels were positively related when the subject group averaged 35 aerobic points per week. The correlation of .58 was statistically significant.

2. Aerobic activity levels and HDL levels were not related when the subject group averaged 50 aerobic points per week. The correlation of .08 was not statistically significant.

3. Changes in aerobic activity levels were positively

related to changes in HDL levels during the 12-week study period. The correlation of .47 was statistically significant.

4. The general relationship between aerobic activity levels and HDL levels was significant at low to moderate activity levels (0 to 40 aerobic points per week). At high levels (exceeding 60 aerobic points per week), further increases in activity levels were not associated with increases in HDL levels.

RECOMMENDATIONS

On the basis of the findings and conclusions of this study, the researchers recommend that the Air University continue to maintain the present Long Haul Program. Its 30 aerobic-points-per-week requirement is an appropriate fitness standard.

The researchers also make the following recommendations for additional studies:

1. Further research should be conducted using a larger number of sedentary subjects (e.g. 100 sedentary subjects as a control group and another 100 sedentary subjects where their aerobic activity levels were raised to 30 to 60 aerobic points per week).

2. Further research in this area should include procedures to determine test reliability for the HDL/cholesterol measurement.

3. Further research in this area should include weekly analysis of HDL/cholesterol levels during the study period for subjects whose activity level remains constant.

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APPENDIX A

Cardiovascular Risk Factors

Appendix A
Cardiovascular Risk Factors

Displayed in the following table are the thirteen factors indorsed by the American Heart Association (1:2-20) as contributing to an individual's overall cardiovascular disease risk. The relative risk points associated with each factor were determined through research conducted at the United States Sports Academy, Preventive Medicine Center (16:1-41).

Risk Factor	Range of Values	Associated Risk Points
Chol Ratio	< 4.5 - 9.5 *	0 - 10
Total Chol	140 - 280 mg/dl	(unassigned)
Smoking	0 - 40 cig/day	0 - 8
Personal History	None - Recent M.I.	0 - 8
Cardio Fitness	Superior - Poor	0 - 6
Psycho Stress	None - Extreme	0 - 4
Age	< 20 - 60 yrs	0 - 4
Diastolic BP	< 80 - 112 mm	0 - 4
Systolic BP	< 124 - 164 mm	0 - 4
Family History	None - Early Death	0 - 4
Body Fat	< 16% - 36% *	0 - 4
Blood Glucose	< 120 - 150 mg/dl	0 - 3
Serum Triglyceride	< 100 - 260 mg/dl	0 - 2

* Note: Expected range in values for males 30 - 39 years old.

Table A-1. Cardiovascular Risk Factors

APPENDIX B

Survey # 1/Aerobic Activity Chart

Appendix B
Survey # 1/Aerobic Activity Chart

AU SCN 87-4 (EXP DATE 15APR87

ACSC CARDIOVASCULAR RISK FACTOR/AEROBIC ACTIVITY LEVEL SURVEY

1. Back in August, you were one of 391 ACSC students who participated in a cardiovascular risk factor (CRF) assessment project. As a follow-on to that test we are conducting a research project to investigate a possible correlation between aerobic exercise and a primary risk factor, high density lipoprotein (HDL). Your name was randomly picked to assist us in gathering some additional needed information.

2. The attached survey will provide us with the information we need to establish a preliminary relationship between CRFs and certain behavior patterns. We also invite you to participate in a second CRF assessment in late November. Having two sets of data (CRFs and aerobic activity) from each of you will significantly improve the statistical validity of our results. Please answer this survey and keep records of your current aerobic activity as accurately as possible. Your actual data is important to us regardless of your HDL level or your compliance with ACSC Long Haul requirements.

3. This project is one of the first to attempt to positively relate aerobic exercise with HDL. It is being enthusiastically sponsored by Dr. Neubauer of England AFB, who plans on publishing the results in a national medical journal. Of course your individual responses to this survey and to any further tests will be kept confidential. Thank you for your interest and cooperation in this important study. There will be a short briefing in the main auditorium at 1200 this Thursday (23 October) to answer any questions concerning the project or survey. You may turn in the survey at that time. If you are unable to make this briefing, please return the completed survey to Maj Stafford/Seminar 25 or Maj Prewitt/Seminar 11 by 24 October, 1986.

Appendix B

INSTRUCTIONS: Except as noted otherwise, circle the letter corresponding to the most correct answer. All questions refer to your behavior/activities during the period 1 July through 31 August, 1986.

1. My average weekly alcohol consumption was (1 drink = 1 beer = 1 glass of wine = 1 oz. hard liquor):

A. None
B. 1 - 4
C. 5 - 8
D. 9 - 12
E. 13 or more

2. My average daily cigarette consumption was:

A. None
B. Less than 1 (or smoke cigars/pipe)
C. 1 - 9
D. 10 - 20
E. 21 or more

3. (USAF officers only) My time for the last aerobic fitness test (1.5 mile) was:

A. Don't know/Haven't actually run one in last 12 months
B. More than 15:30
C. 15:30 - 13:01
D. 13:00 - 11:00
E. Less than 11:00

4. My average number of aerobic exercise sessions (must exceed 15 minutes per session) per week was:

A. Less than 1
B. 1 - 2
C. 3 - 4
D. 5 - 6
E. 7 or more

Appendix B

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5. My average weekly aerobic exercise consisted of the following: (insert appropriate distance/pace)

Event	Distance	Pace
Walking (add only walks of one mile or more)	_____ mi.	N/A
Running/Jogging	_____ mi.	_____ mins/mi.
Bicycling	_____ mi.	_____ mins/mi. or _____ mph
Swimming	_____ yds	_____ sec/100 yds
Basketball, Racquetball Handball, Squash	_____ hours	N/A
Tennis	_____ hours	N/A
Other (specify) _____	_____ ____	_____ _____

Note: leave question # 6 blank if "other" aerobic exercise was a significant part of your exercise program.

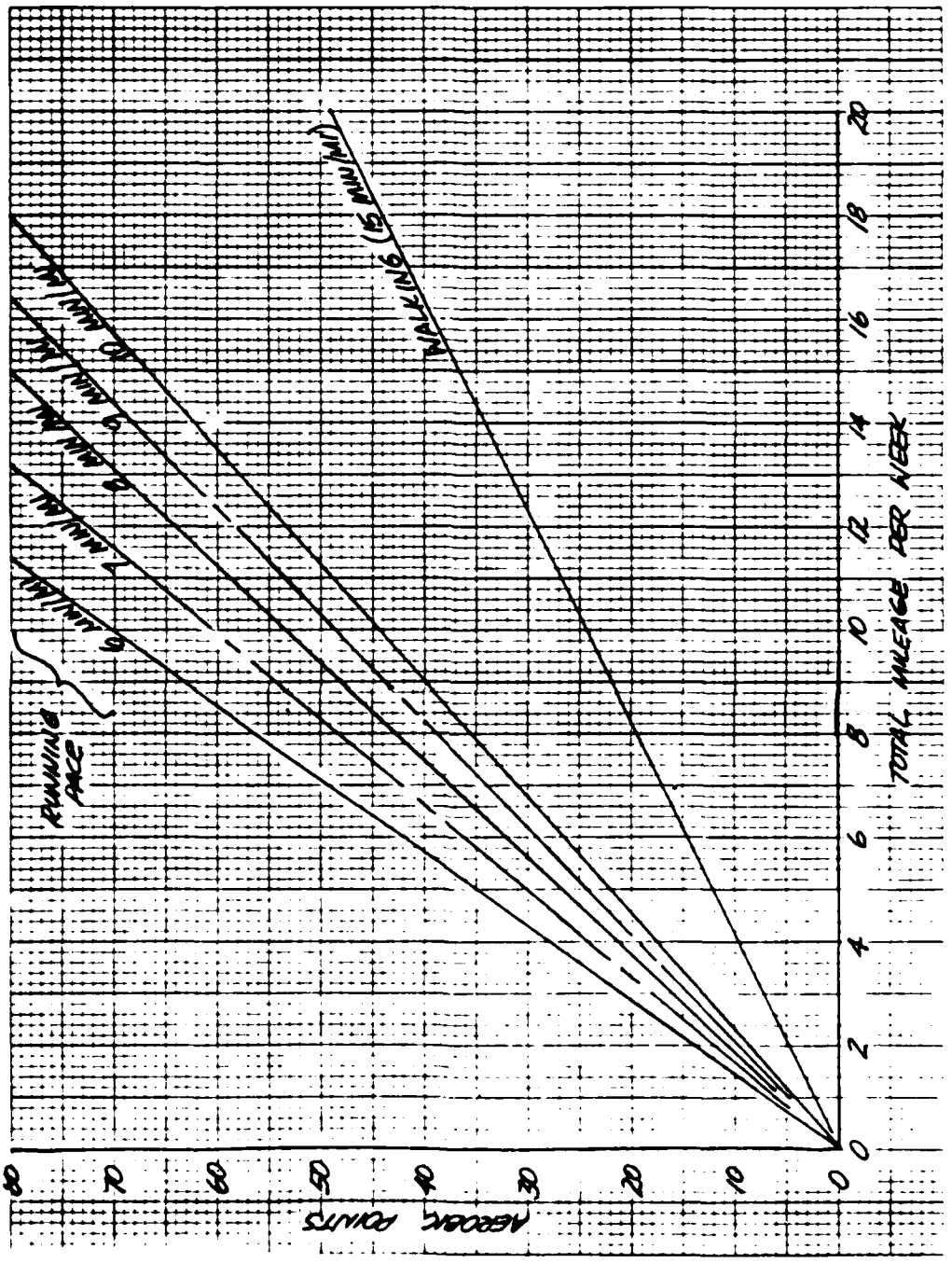
6. Using the attached charts and the data from question 5, my average total weekly aerobic exercise points were: _____ points.

Note: If specific exercise duration exceeds the maximum value on the charts, divide the duration by two, extract appropriate points, then multiply by two. (Example--If you run 30 miles/week at 9 min/mile: 15 miles at that pace = 73 points, so 30 miles = 146 points.)

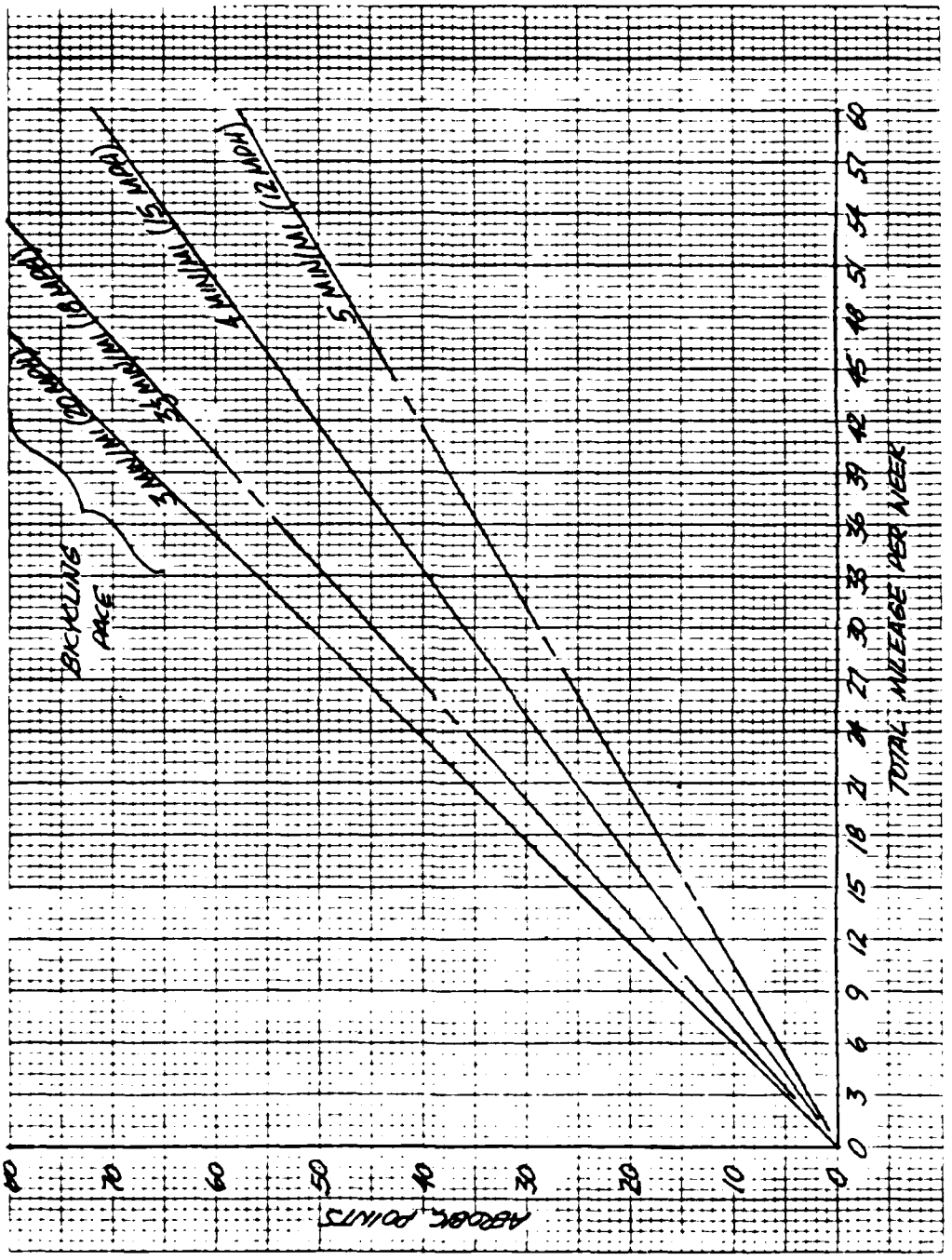
7. I am willing to accurately record my aerobic points for the next two months and take a second CRF blood test in November.

A. Yes
B. No

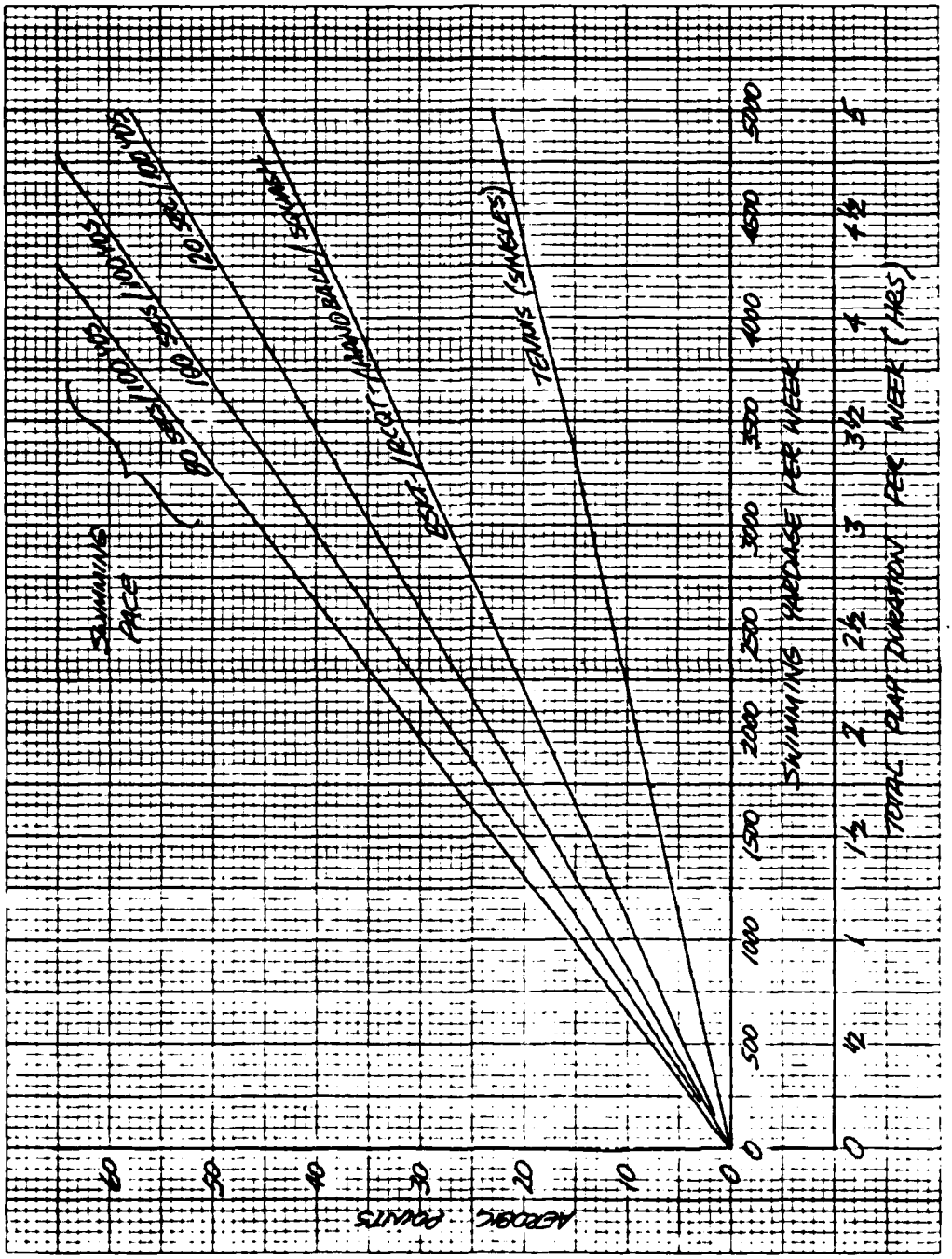
Appendix B


WALKING (15 min/mi)
9 min/mi
8 min/mi
7 min/mi
6 min/mi
5 min/mi
PACE
TOTAL MILEAGE PER WEEK
0
2
4
6
8
10
12
14
16
18
20
AEROBIC POINTS
80
70
60
50
40
30
20
10
0

Appendix B


BICKLING PACE
5 MINIMI (12 MPH)
4 MINIMI (15 MPH)
3 MINIMI (20 MPH)
ABOOK POINTS
TOTAL MILEAGE PER WEEK

Appendix B


AEROBIC POINTS
0
10
20
30
40
50
60
SWIMMING PACE (MIN/100 YDS)
TENNIS (SINGLES)
TENNIS (DOUBLES)
JOGGING (10 MIN/MILE)
SWIMMING (2 MIN/100 YDS)
SWIMMING YARDAGE PER WEEK
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
TOTAL PLAY DURATION PER WEEK (HRS)
0
½
1
1½
2
2½
3
3½
4
4½
5

APPENDIX C

Rapid Stat Procedure

Appendix C
Rapid Stat Procedure

INSTRUCTIONS - Read Carefully

PRODUCT NOS. 9683-441001, -441027, -441043, -441100, -441092

LANCER®

U.S. Patent 4,226,713; 4,239,849 Patent Pending

HDL/Cholesterol
Rapid Stat® Diagnostic Kit

Precipitant Reagents (441027, 441043), Cholesterol Rapid Stat Kit (441100) and Standards Set (441092)

For the Separation of HDL/Cholesterol in Serum and the Quantitative Colorimetric Determination of Total and HDL Cholesterol in Serum.

PRODUCT DESCRIPTION AND INTENDED USE

The HDL/Cholesterol Rapid Stat® Kit is a set of reagents and standards for performing the quantitative determination of total cholesterol and HDL cholesterol in serum. The kit consists of three reagents: Enzyme Reagent, Phenol Reagent and Precipitant Reagent, and three cholesterol standards for direct use or in preparing a calibration graph. The Cholesterol Rapid Stat® Kit consists of Enzyme Reagent, Phenol Reagent and two cholesterol standards.

SUMMARY AND EXPLANATION OF TEST

The importance of cholesterol (and cholesterol esters) has long been recognized in clinical medicine and therefore has been widely studied. Many types of procedures have been utilized — gravimetric, nephelometric, chromatographic, colorimetric, fluorometric, turbidimetric. However, spectrophotometric methods are most frequently used. The Liebermann-Burchard reaction is a classical procedure for determining cholesterol in an acetic acid — acetic anhydride — sulfuric acid system.[1] The procedure of Abell[2] *et al*, widely accepted as the reference method, employs solvent extraction of the cholesterol and cholesterol esters followed by hydrolysis prior to color development.

Enzymatic methods[3] utilizing Cholesterol Oxidase and Cholesterol Esterase have recently become available and have been combined eliminating the need for any extraction procedure. The Rapid Stat procedure utilizes both enzymes in conjunction with the peroxidase/phenol/4-aminoantipyrine system outlined by Trinder.[4] The chemical reactions are shown in the "PRINCIPLE OF THE PROCEDURE" section.

Total circulating cholesterol is composed of two major fat packets — the high-density lipoprotein (HDL) fraction and the low- or very low-density lipoprotein (LDL, VLDL) fractions.

Various lipoprotein fractions can be separated electrophoretically. A quantitative lipoprotein profile can be obtained by ultracentrifugation or by selective precipitation of the LDL and VLDL fraction. The Rapid Stat procedure utilizes a rapid, efficient and stable magnesium/phosphotungstate precipitant reagent.[5] The HDL cholesterol fraction present in the supernatant is then analyzed with our totally enzymatic cholesterol reagent.

CLINICAL SIGNIFICANCE

Many experimental and epidemiological studies have shown an association between serum lipids and atherosclerosis. Cholesterol levels and dietary intake have often been related to atherosclerosis as well as resultant stroke, cardiovascular disease, etc.

Additionally, elevated cholesterol levels are seen with various lipid diseases, hyperlipemias and hyperlipoproteinemias. Commonly observed secondary causes of elevated cholesterol levels are diabetes mellitus, nephrotic syndrome, hypothyroidism, and hepatitis in its early stages.

The HDL cholesterol fraction appears to be much more closely related to coronary heart disease than are total cholesterol levels. Barr[6] *et al*, observed an inverse relationship between HDL levels and the incidence of heart disease in male patients. Additional recent studies have shown the value of HDL levels as playing a predictive/protective role in coronary heart disease.[7, 8, 9, 10] Therefore, this determination has the potential of becoming an excellent prognostic test for detection of high risk individuals.

PRINCIPLE OF THE PROCEDURE

1. Serum $\xrightarrow[\text{Reagent}]{\text{Precipitant}}$ HDL fraction (Supernatant) + (LDL + VLDL) (Precipitate)

2. HDL Cholesterol or Serum Cholesterol + Cholesterol Esters $\xrightarrow[\text{Esterase}]{\text{Cholesterol}}$ Free Cholesterol

3. Free Cholesterol $\xrightarrow[\text{Cholesterol Oxidase}]{O_2}$ Cholest-4-en-3-one + H_2O_2

4. H_2O_2 + phenol + 4 aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + H_2O (pink)

REAGENTS

The HDL/Cholesterol Rapid Stat® Kit contains sufficient reagents and standards for performing 50 determinations as follows:

1. **Precipitant Reagent** (25 mL) containing: $MgCl_2 \cdot 6H_2O$, 11 g/L; Phosphotungstic Acid, 4.4 g/L.
2. **Enzyme Reagent*** (2 x 1.36 gm) containing (after reconstitution): Peroxidase (horseradish), > 40 x 10^3 U/L; Cholesterol Oxidase (bacterial), > 100 U/L; Cholesterol Esterase (animal), > 50 U/L; 4-aminoantipyrine, 0.3 g/L. Also contains a stabilizer and buffers.
3. **Phenol Reagent** (100 mL) containing: Phenol, 2 g/L. Also contains stabilizers.
4. **Cholesterol Standard** 30 mg/dL (0.78 mmol/L) (5 mL) containing: Cholesterol, 30 mg/dL; Surfactant; Stabilizer.
5. **Cholesterol Standard** 200 mg/dL (5.2 mmol/L) (5 mL) containing: Cholesterol, 200 mg/dL; Surfactant; Stabilizer.
6. **Cholesterol Standard** 500 mg/dL (13 mmol/L) (5 mL) containing: Cholesterol, 500 mg/dL; Surfactant; Stabilizer.

The bulk Precipitant Reagents (Product Nos. 9683-441027 and 9683-441043) have the same exact formulation as the kit reagent and contains sufficient material for 200 and 480 precipitations respectively.

The Cholesterol Rapid Stat Kit contains sufficient reagents and standards for performing 50 determinations as follows:

1. **Enzyme Reagent*** (2 x 1.36 gm) containing (after reconstitution): Peroxidase (horseradish), > 40 x 10^3 U/L; Cholesterol Oxidase (bacterial), > 100 U/L; Cholesterol Esterase (animal), > 50 U/L; 4-aminoantipyrine, 0.3 g/L. Also contains a stabilizer and buffers.
2. **Phenol Reagent** (100 mL) containing: Phenol, 2 g/L. Also contains stabilizers.
3. **Cholesterol Standard** 200 mg/dL (5.2 mmol/L) (5 mL) containing: Cholesterol, 200 mg/dL; Surfactant; Stabilizer.
4. **Cholesterol Standard** 500 mg/dL (13 mmol/L) (5 mL) containing: Cholesterol, 500 mg/dL; Surfactant; Stabilizer.

*Lot-to-lot variations in enzyme activity may occur; however, in no case will such variations affect or alter test results.

Appendix C

Warnings/Precautions

For In Vitro Diagnostic Use

CAUTION: The physiological properties of these reagents are unknown. Therefore, use good chemical handling procedures.

The standards contain propanol and therefore are flammable; do not expose to heat, spark or open flame!

Working Reagent Preparation

To prepare Working Cholesterol Reagent, bring stock reagents to room temperature, add 50.0 mL of Phenol Reagent to the 2 oz. bottle containing the Enzyme Reagent. Allow to stand 10 minutes, then swirl to mix completely. If more than 1 bottle of Working Cholesterol Reagent is being prepared at the same time, the reagent can be pooled. A standard must be run on each bottle (single or pooled) of prepared Working Cholesterol Reagent. Working Cholesterol Reagent is stable 60 days refrigerated.

Storage and Stability

Upon receipt the kit should be stored in the refrigerator. DO NOT FREEZE. After opening the kit the Enzyme Reagent, Phenol Reagent and standards must be stored under refrigeration at 2-8°C. The Precipitant Reagent may be stored at room temperature (18-25°C). If the standards have been subjected to freezing temperatures, the cholesterol may form a crystalline precipitate. If this should occur, place tightly capped standard in a 37°C incubator overnite, then shake to resolubilize.

All reagents should be clear. Should any reagent precipitate or show signs of microbial growth, discard it. The Working Cholesterol Reagent should be almost colorless — it may have a pink cast as it ages.

SPECIMEN COLLECTION AND PREPARATION

HDL Cholesterol: The specimen must be collected from a fasting patient, the fast being a full 14 hour fast.[1] The patient should have been on a full ethnic diet for several days prior to the fast and subsequent blood sampling. The serum can be stored at room temperature up to 3 days or at 2-8°C up to 6 days. Refrigerated or frozen specimens which have completely thawed require a full 24-30 hour period to fully resolubilize the lipoproteins. If these specimens are analyzed before the required equilibration period, the results will be artificially low.

Plasma collected in EDTA is suitable for analysis (results will tend to be 1.5 mg/dL [0.039 mmol/L] lower than serum.) Do not use heparinized plasma or plasma collected in other coagulants.

Total Cholesterol: The specimen must be collected from a fasting patient, the fast being a full 14 hour fast. The patient should have been on a full ethnic diet for several days prior to the fast and subsequent blood sampling. The Cholesterol in the specimen is stable up to 6 days at 2-8°C.

PROCEDURE

Materials Required

In addition to the HDL/Cholesterol or Cholesterol Rapid Stat Kits, the following items will be needed to perform the test:

1. Conical centrifuge tubes (12 mL glass is suitable).
2. Glass test tubes (13 x 100 mm are suitable).
3. Accurate pipettes (such as Oxford® brand Samplers®) capable of delivering 50.0 mL, 2.0 mL, 0.50 mL, 0.10 mL and 0.02 mL.
4. Centrifuge capable of obtaining 1000 x g (non-refrigerated).
5. Incubation bath set at 37°C.
6. Spectrophotometer or colorimeter capable of reading absorbance at 510 nm.

Performance of Test (HDL Cholesterol)

A. Preparation of HDL fraction

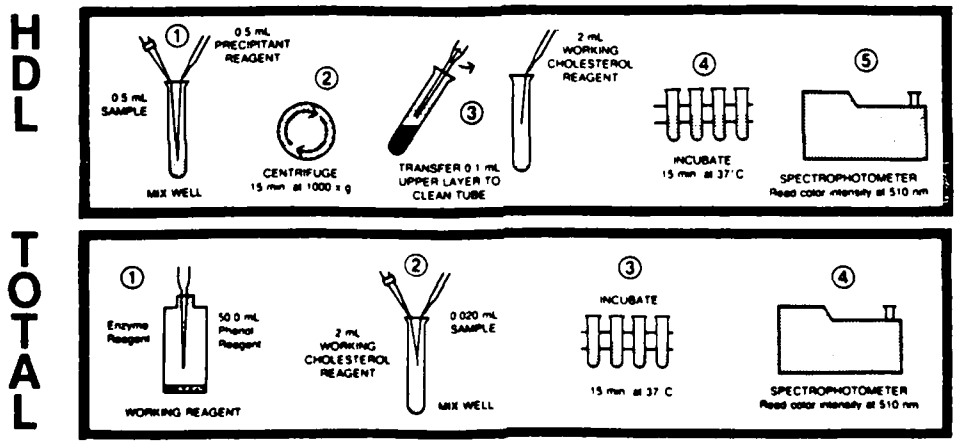
1. Pipet 0.50 mL serum into labelled conical centrifuge tube.
2. Add 0.50 mL Precipitant Reagent. Mix well.
3. Centrifuge all tubes at 1000 x g (full speed for most bench centrifuges) for 15 minutes. (See note 4.)
4. Carefully remove clear supernatant fraction. (See note 5.) Transfer to a properly labelled tube marked HDL fraction. (If supernatant is turbid, see note 1).

B. Procedure for HDL Cholesterol determination

1. Prepare Working Cholesterol Reagent as indicated in WORKING REAGENT PREPARATION section.
2. Label test tubes, one for the reagent blank, one for standard and one for each patient.
3. (a) To the tube designated reagent blank, add 0.100 mL of deionized water.
   (b) To the tube designated standard, add 0.100 mL of the cholesterol standard, 30 mg/dL (0.78 mmol/L). (see note 7.)
   (c) To each patient tube, add 0.100 mL of the HDL fraction prepared in section A above.
4. Add 2.0 mL of Working Cholesterol Reagent into each tube. Mix well.
5. Incubate rack of tubes in 37°C bath for 15 minutes.
6. Remove all tubes from incubation bath. Cool in room temperature (18-25°C) water bath for 1-2 minutes.
7. With the wavelength of the spectrophotometer set at 510 nm, set the absorbance of the instrument to zero with the Reagent blank. Then read and record the absorbances of all standard and patient tubes within 30 minutes. To compute HDL cholesterol concentration, see "CALCULATION OF HDL CHOLESTEROL CONCENTRATION" section.

Performance of Test (Total Cholesterol)

1. Prepare Working Cholesterol Reagent as indicated in WORKING REAGENT PREPARATION section.
2. Label test tubes, one for the reagent blank, one for standard and one for each patient.
3. (a) To the tube designated reagent blank, add 0.020 mL of deionized water.



Appendix C

(b) To the tube designated standard, add 0.020 mL of the cholesterol standard, 200 mg/dL (5.2 mmol/L). (see note 7.)
(c) To each patient tube, add 0.020 mL of whole serum.

4. Add 2.0 mL of Working Cholesterol Reagent into each tube. Mix well.
5. Incubate rack of tubes in 37°C bath for 15 minutes.
6. Remove all tubes from incubation bath. Cool in room temperature (18-25°C) water bath for 1-2 minutes.
7. With the wavelength of the spectrophotometer set at 510 nm, set the absorbance of the instrument to zero with the Reagent Blank. Then read and record the absorbances of all standard and patient tubes within 30 minutes. To compute total cholesterol concentration, see "CALCULATION OF TOTAL CHOLESTEROL CONCENTRATION" section.

Calibration

HDL Cholesterol

This procedure follows Beer's law (linear relationship between concentration and absorbance, intercept = 0) over the range 0-150 mg cholesterol/dL (0-3.9 mmol/L). Individual spectrophotometers and colorimeters may vary in their characteristics, making it best that these instruments be calibrated. For most specimens the use of the Blank and 30 mg/dL (0.78 mmol/L) standard may be used with no other standard solutions over the range in which Beer's law is known to hold for the particular instrument.

The following chart shows the proper ratios of the standards provided in the kit to use in preparing a series of cholesterol standards of various concentrations over the 0 to 150 mg/dL (0-3.9 mmol/L) range. **Do not dilute standards with water or saline.** Analyze in same manner as "Procedure for HDL Cholesterol determination." (Deionized water may be used as a zero standard.) (see note 7.)

Cholesterol Concentration (mg/dL)	Cholesterol Concentration (mmol/L)	Volume of 30 mg/dL (0.78 mmol/L) Standard (mL)	Volume of 200 mg/dL (5.2 mmol/L) Standard (mL)
30	0.78	only	—
50	1.30	0.075	0.010
80	1.56	0.140	0.030
100	2.60	0.100	0.070
115	2.99	0.050	0.050
130	3.38	0.070	0.100
150	3.90	0.050	0.120

Total Cholesterol

This procedure follows Beer's Law over the range 0-500 mg cholesterol/dL (0-13 mmol/L). For most specimens the use of the Blank and 200 mg/dL (5.2 mmol/L) standard may be used with no other standard solutions over the range in which Beer's law is known to hold for the particular instrument.

The following chart shows the proper ratios of the standards provided in the kit to use in preparing a series of cholesterol standards of various concentrations over the 0 to 500 mg/dL (0-13 mmol/L) range. **Do not dilute standards with water or saline.** Analyze in same manner as "Performance of Test — Total Cholesterol". (Deionized water may be used as a zero standard.) (see note 7.)

Cholesterol Concentration (mg/dL)	Cholesterol Concentration (mmol/L)	Volume of 30 mg/dL (0.78 mmol/L) Standard* (mL)	Volume of 200 mg/dL (5.2 mmol/L) Standard (mL)	Volume of 500 mg/dL (13 mmol/L) Standard (mL)
100	2.60	0.100	0.070	—
200	5.20	—	only	—
300	7.80	—	0.100	0.050
400	10.4	—	0.050	0.100
500	13.0	—	—	only

*Available in Cholesterol Standards Set, Product No. 9683-441092.

Quality Control

HDL Cholesterol

Most lyophilized sera are unsuitable for the Quality Control of the HDL Cholesterol due to changes in the lipoprotein structure. It is therefore recommended that the Lancer HDL/Cholesterol Control Serum (Product No. 8883-441050) be used.

Total Cholesterol

The use of assayed serum controls, in both the normal and abnormal ranges, is helpful in monitoring the performance of the procedure. It is good practice to run a normal and abnormal control with each batch of samples. The value for each control should fall within the manufacturer's stated acceptable range based on comparable enzymatic methods.

RESULTS

Calculation of HDL Cholesterol Concentration

In the range in which Beer's law has been demonstrated to hold for the instrument used and when the 30 mg/dL (0.78 mmol/L) Cholesterol standard is used:

$$\text{HDL Cholesterol Concentration of Unknown} = \frac{\text{A unknown}}{\text{A standard}} \times 30 \frac{\text{mg}}{\text{dL}} (0.78 \text{ mmol/L}) \times 2$$

Where A = absorbance

The factor of 2 will correct for the dilution of the sample with the Precipitant Reagent.

Sample Calculation

A unknown = 0.196
A standard = 0.245

$$\text{HDL Cholesterol Concentration of Unknown} = \frac{0.196}{0.245} \times 30 \frac{\text{mg}}{\text{dL}} (0.78 \text{ mmol/L}) \times 2 = \underbrace{48 \text{ mg/dL}^*}_{\text{HDL Cholesterol}} (1.25 \text{ mmol/L})$$

To arithmetically estimate the LDL and VLDL values, see note 6.

*To convert from mg/dL to SI units, multiply by 0.026 (e.g. 48 mg/dL = 1.25 SI units [mmol/L]).

Calculation of Total Cholesterol Concentration

In the range in which Beer's law has been demonstrated to hold for the instrument used, and when the 200 mg/dL (5.2 mmol/L) cholesterol standard is used:

$$\text{Total Cholesterol Concentration of Unknown} = \frac{\text{A unknown}}{\text{A standard}} \times 200 \frac{\text{mg}}{\text{dL}} (5.2 \text{ mmol/L})$$

Where A = absorbance

Sample Calculation

A unknown = 0.536
A standard = 0.346

$$\text{Total Cholesterol Concentration of Unknown} = \frac{0.536}{0.346} \times 200 \frac{\text{mg}}{\text{dL}} (5.2 \text{ mmol/L}) = 310 \frac{\text{mg}}{\text{dL}} \text{ cholesterol}^* (8.06 \text{ mmol/L})$$

*To convert from mg/dL to SI units, multiply by 0.026 (e.g. 310 mg/dL = 8.06 SI units [mmol/L]).

NOTES AND PRECAUTIONS

1. Lipemic specimens, and on occasion a clear specimen, may give a supernatant that is turbid. If you have the capability, centrifuge an additional 10-15 minutes up to 12,000 g. Otherwise, dilute the specimen 1:1 with saline and repeat the precipitation, multiplying the final HDL result by 2.
2. Specimens which are severely lipemic may give a compact precipitate (appearance of scum) on the top with a clear subnatant instead of a clear supernatant and a "button". Then carefully bypass the "pedacle" on top and sample from the subnatant.
3. Specimens which have a Total Cholesterol greater than 500 mg/dL (13 mmol/L) should be diluted 1:1 with saline and rerun. Multiply the final total cholesterol result by 2.
4. To convert r.p.m.'s to g, use the following formula:

$$g = 28.38 (R) \left(\frac{N}{1000}\right)^2 \quad \text{Where } R = \text{radius of centrifuge head in inches}, \; N = \text{r.p.m.'s}$$

Appendix C

5. Remove supernatant immediately after centrifugation. If the supernatant is allowed to stand on top of the button for an excessive period of time (more than 30 minutes), the cholesterol value in the HDL fraction will be artificially elevated.
6. $C_{VLDL} = TG/5$
   $C_{LDL} = C_{total} - C_{HDL} - TG/5$
   Where: C = concentration of cholesterol in mg/dL
   TG = serum triglyceride concentration in mg/dL

   These equations are *fairly* accurate for specimens in which the triglyceride concentrations are no more than 400 mg/dL (4.52 mmol/L) and which are *not* from persons having type III hyperlipoproteinemia.[16]
7. It is extremely important to take care to accurately pipette the standards because they contain a surfactant and an alcohol. It is therefore suggested that both the standards and samples be added with the "reverse" technique. This technique is only possible when a two-stop piston pipette with disposable tips (such as Oxford® brand Samplers®) is used. To fill the disposable tip, the piston is depressed all of the way down to the second stop, dipped below the surface of the liquid and allowed to rise gradually all the way up. Repeat the up and down motion of the piston to "pre-wet" the tip. The outside of the tip is carefully wiped with clean absorbent paper, being careful to avoid removal of fluid from within the tip. The tip is then placed *near* the bottom of a clean test tube and the piston pushed down to the first stop. This will cause the volume of fluid stated on the pipette to be displaced. The point of the tip is then touched to the inner wall of the test tube in order to transfer any droplets from the tip to the tube.

   Additionally, the standard, since it contains alcohol, should be protected from evaporation. This can be accomplished by "squeezing" only enough out of the bottle to complete the test, immediately pipetting, and discarding the waste.

EXPECTED VALUES

Since many factors (age, sex, race, diet) seem to affect HDL/Cholesterol and Total Cholesterol levels, it is best that each laboratory determine its own normal range.

HDL/Cholesterol

Using the Rapid Stat procedure for hospitalized, caucasian patients: for 25 male patients, average age of 53, the mean HDL/Cholesterol was 44.9 mg/dL (1.17 mmol/L) (range = 35-64 mg/dL, 0.91-1.66 mmol/L); for 24 female patients, average age of 59, the mean HDL/Cholesterol was 56 mg/dL (1.46 mmol/L) (range = 37-74 mg/dL, 0.96-1.92 mmol/L).

The following normal population ranges have been suggested:[13] male, 29-61 mg HDL Cholesterol/dL (0.75-1.59 mmol/L); female, 38-75 mg HDL Cholesterol/dL (1.0-1.95 mmol/L). Values below limits are associated with a higher than average risk of coronary heart disease (CHD). Values above 55 mg/dL (1.43 mmol/L) are associated with a lower than average risk of CHD.

Total Cholesterol[13]

	Male		Female	
Age	mg/dL	mmol/L	mg/dL	mmol/L
< 20	< 180	< 4.7	< 180	< 4.7
20-30	140-260	3.6-6.8	140-240	3.6-6.2
30-40	140-280	3.6-7.3	140-240	3.6-6.2
40-50	140-280	3.6-7.3	150-280	3.9-7.3
> 50	140-280	3.6-7.3	180-330	4.7-8.6

PERFORMANCE CHARACTERISTICS

Accuracy

HDL Cholesterol

Precipitations were done on 30 patient sera and the HDL fraction obtained using both the Heparin/Mn^{++} method and the Rapid Stat Precipitant Reagent. These HDL fractions were then analyzed with this enzymatic cholesterol reagent on the ABA-100®. The following regression equation was established:

$y = 0.98x - 9.217$ mg/dL ($- 0.24$ mmol/L) y = Lancer procedure, x = Heparin/Mn^{++} procedure

When the 9 mg/dL (0.234 mmol/L) bias associated with the enzymatic determination of HDL Cholesterol prepared by the Heparin/Mn^{++} procedure[14, 15] is corrected, the correlation between the two precipitation methods is 0.981

Total Cholesterol

Twenty patient sera were analyzed both on an ABA-100 using the Rapid Stat Cholesterol Enzyme Reagent and with Hycel reagents on the Hycel Super 17™. These results show that the Hycel reagents give results approximately 5% higher than the Lancer values.

All commonly used laboratory control sera have been analyzed with the Rapid Stat Cholesterol Reagents. These analyses have all been within the manufacturer's stated range based on comparable enzymatic methods.

Precision

Inter-run precision: Samples were taken from a serum pool and analyzed over a 40 day period for HDL/Cholesterol and Total Cholesterol. The results are tabulated below:

Test	No. of Samples	Mean mg/dL	Mean (mmol/L)	S.D. mg/dL	S.D. (mmol/L)	$\left(\frac{\text{S.D.}}{\text{Mean}} \times 100\right)$ C.V.
HDL Cholesterol	10	43	1.12	± 1.03	± 0.027	2.4%
Total Cholesterol	16	248	6.45	± 5.2	± 0.14	2.1%

HDL Cholesterol

Intra-run precision: A serum pool was analyzed for HDL Cholesterol ten times per day on three separate days. The mean, standard deviation and C.V. were calculated for each day and then averaged.

Sample	Mean HDL Cholesterol mg/dL	(mmol/L)	S.D. mg/dL	(mmol/L)	$\left(\frac{\text{S.D.}}{\text{Mean}} \times 100\right)$ C.V.
Serum pool	42.9	1.115	± 0.41	± 0.01	0.95%

Total Cholesterol

Intra-run precision: Using a single batch of the prepared Working Cholesterol reagent, the 250 mg/dL (6.5 mmol/L) Cholesterol Standard and a control serum were each analyzed 10 times giving the following results:

Sample	Average Absorbance	S.D.	C.V.
250 mg/dL (6.5 mmol/L) Cholesterol Standard	0.397	± 0.001	0.3%
Control Serum	0.360	± 0.002	0.6%

Sensitivity

The sensitivity of this method for cholesterol concentration was calculated according to the following equation:

$a = \frac{A}{CL}$ Where: a = absorptivity, A = absorbance, C = concentration (g/L), L = light path (cm)

Thus, the sensitivity of this method, expressed as absorptivity, is 17.5 L/gm-cm. The 200 mg/dL (5.2 mmol/L) standard gives a typical absorbance of 0.346 ± 0.015 for a 1 cm cell.

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M- 29-61

F 38-75

L 253-0

LANCER

Athy, Co. Kildare, Ireland.

020-120
1947302
Rev. July, 1981
Printed in Ireland

APPENDIX D

Health Assessment Worksheet

Appendix D
Health Assessment Worksheet

ACSC HEALTH ASSESSMENT WORKSHEET

Please record the following information and deposit in the specially marked container located in the lounge. **All information collected will be confidential.** Blood analysis results and this worksheet will be returned to you later.

1. **NAME:** XXX XXXXXX DATE: 28 AUG 86
2. **STUDENT NO.:** XXXX
3. **SEMINAR NO.:** XX
4. **AGE:** 38
5. **HEIGHT:** 73
6. **WEIGHT:** 161
7. **SYSTOLIC BLOOD PRESSURE:** 122
8. **DIASTOLIC BLOOD PRESSURE:** 90
9. **RESTING HEART RATE (PULSE):** 60
10. **LIST ANY MEDICATION YOU ARE TAKING:** ADVIL

APPENDIX E

Sample Lab Sheet

Appendix E
Sample Lab Sheet

APPENDIX F

Survey # 2

Appendix F
Survey # 2

NAME_____ SEMINAR_____

ACSC CARDIOVASCULAR RISK FACTOR/AEROBIC ACTIVITY LEVEL SURVEY # 2

THANK YOU FOR AGREEING TO PARTICIPATE IN THIS STUDY. YOU ARE SCHEDULED TO HAVE A FINAL BLOOD SAMPLE DRAWN ON TUESDAY, THE 25TH OF NOVEMBER, 1986 AT _____. LOCATION WILL BE IN THE ACSC SNACK BAR.

YOU WILL NEED TO FAST FOR 12 TO 14 HOURS BEFORE THE TIME NOTED ABOVE. WATER IS THE ONLY ITEM ALLOWED. YOU ALSO NEED TO REFRAIN FROM ANY ALCOHOL FOR 72 HOURS PRIOR TO THIS TIME.

PLEASE COMPLETE AND BRING THIS SLIP WITH YOU FOR THE BLOOD TEST.

AGE_____HEIGHT_____ WEIGHT_____

INSTRUCTIONS: Except as noted otherwise, circle the letter corresponding to the most correct answer. All questions refer to your behavior/activities during the period 29 September, 1986 through the present.

1. My average weekly alcohol consumption was (1 drink = 1 beer = 1 glass of wine = 1 oz. hard liquor):

A. None
B. 1 - 4
C. 5 - 8
D. 9 - 12
E. 13 or more

2. My average daily cigarette consumption was:

A. None
B. Less than 1 (or smoke cigars/pipe)
C. 1 - 9
D. 10 - 20
E. 21 or more

3. My time for the last aerobic fitness test (1.5 mile) was:

A. Don't know/Haven't actually run one in last 12 months
B. More than 15:30
C. 15:30 - 13:01
D. 13:00 - 11:00
E. Less than 11:00

Appendix F

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4. My average number of aerobic exercise sessions (must exceed 15 minutes per session) per week was:

A. Less than 1
B. 1 - 2
C. 3 - 4
D. 5 - 6
E. 7 or more

5. My use of medication during the last 30 days included:

A. No use of medication
B. Medication as noted

---

6. My weekly Aerobic Points (via Longhaul Procedures) were as follows:

Week Beginning 29 SEP _____

6 OCT _____

13 OCT _____

20 OCT _____

27 OCT _____

3 NOV _____

10 NOV _____

17 NOV _____

THANKS AGAIN! WE WILL SEE YOU THE MORNING OF THE 25TH OF NOVEMBER. RESULTS OF THE BLOOD TEST WILL BE DISTRIBUTED TO YOU AS SOON AS POSSIBLE.

DAVID PREWITT & KEN STAFFORD
PROJECT COORDINATORS

APPENDIX G

Correlation Matrices

Appendix G
Correlation Matrices

	WT	CHOL	TRIG	HDL	RATIO	ALCOHOL	CIG	# SES	# PTS
CHOL	0.13								
TRIG	0.10	0.47							
HDL	0.09	-0.34	-0.20						
RATIO	0.01	0.80	0.38	-0.81					
ALCOHOL	0.09	0.05	0.21	0.18	-0.07				
CIG	0.26	0.26	0.36	-0.04	0.17	0.40			
# SES	0.30	-0.06	-0.07	0.47	-0.33	0.23	0.05		
# PTS	0.18	-0.02	-0.04	0.58	-0.39	-0.02	-0.02	0.78	
FIT LEVEL	-0.04	-0.11	-0.20	0.35	-0.28	-0.04	-0.15	0.42	0.48

Note: Values greater than .23 or less than -.23 are significant at the .05 level of probability

Table G-1. Correlations at Beginning of Study

	WT	CHOL	TRIG	HDL	RATIO	ALCOHOL	CIG	# SES	# PTS
CHOL	0.04								
TRIG	0.14	0.45							
HDL	0.07	0.22	0.05						
RATIO	-0.04	0.22	0.34	-0.54					
ALCOHOL	0.07	0.07	-0.03	-0.15	0.17				
CIG	0.25	0.28	0.28	0.13	0.10	0.29			
# SES	0.01	0.01	-0.02	0.09	-0.09	0.23	0.16		
# PTS	0.04	0.07	-0.20	0.08	-0.03	0.24	-0.16	0.37	
FIT LEVEL	-0.03	-0.41	0.24	-0.14	-0.13	0.11	-0.17	0.09	0.48

Note: Values greater than .23 or less than -.23 are significant at the .05 level of probability

Table G-2. Correlations at End of Study

APPENDIX H

Uncertainty Analysis

Appendix H.
Uncertainty Analysis

The incremental approach provided by Dr. Cooper (6:172-185) posed a problem of precision in analizing the study data. Due to the quite large steps in his aerobic points methodology, nearly identical exercise sessions, individual runs for example, may have been awarded significantly different points. In a typical run (assumed to have been about three miles in a time of about 24 minutes), one second under the critical time (24:00 in this example) would have led to a 21 percent increase in points (14 to 17). Conversely, a one-second increase in elapsed time would have resulted in an 18 percent decrease in points (17 to 14). The researchers concluded, therefore, that the points awarded may have been in error by as much as one-half of this total points jump. For purposes of this analysis, a plus-or-minus 10 percent uncertainty was thereby established for all aerobic points obtained via Dr. Cooper's charts.

On the initial survey, points were obtained via a set of charts that were developed by the researchers using mid-point data from Dr. Cooper's charts (see Appendix B). Since the curves were continuous, the step-wise rounding error noted above was not a factor. Since accuracy of the data, however, depended upon the subjects' recall of their activities prior to arriving at ACSC, the assigned plus-or-minus 10 percent uncertainty still appeared reasonable.

HDL measurement reliabilities were not experimentally assessed during this study. The published inter-run precision of the test is 2.4 percent (18:--; 20:--). Conversations with laboratory technicians suggested, however, that in actual practice, plus-or-minus 5 percent was a more reasonable figure to use (17:--).

Having established these parameter uncertainties, the researchers calculated the uncertainties in each of the derived HDL/points sensitivities in analysis groups A through H. The method described by Kline and McClintock (7:37-39) was used. Essentially it amounted to developing an uncertainty equation which equalled the square root of the sum of squares of the individual parameter uncertainty contributions. These individual terms were, in turn, the partial derivatives of the sensitivity equation with respect to each parameter, times that parameters' uncertainty. The results of this analysis are displayed in Table H-1 and on Figure 1 in chapter four.

Appendix H

Group	A	B	C	D	E	F	G	H
Uncertainty (mg/dl +/-)	.13	.33	N/A	.12	.14	.71	1.0	.23

Table H-1. HDL/Points Sensitivity Uncertainties

APPENDIX I

Raw Data

Appendix I
Raw Data

#	AGE	HT	WT	CHOL	TRIG	HDL	RATIO	ALCO	CIG	FIT	# SES	PTS
1	35	70	187	182	174	62	2.9	11	0	4	5.5	66
2	36	69	172	155	165	48	3.2	15	0	4	3.5	45
3	35	73	224	220	202	38	5.8	0	15	3	3.5	30
4	34	72	195	164	116	46	3.6	0	0	4	3.5	34
5	35	71	198	198	96	58	3.4	15	25	4	3.5	50
6	36	68	167	182	152	32	5.7	11	0	4	1.5	30
7	32	71	145	132	87	40	3.3	3	0	5	3.5	34
8	33	70	175	181	115	64	2.8	3	0	4	3.5	40
9	37	73	202	192	63	34	5.6	0	0	5	3.5	29
10	39	69	170	209	117	42	5.0	0	0		0	0
11	36	66	160	161	62	64	2.5	0	0		5.5	47
12	37	71	195	220	142	46	4.8	7	0	5	3.5	85
13	40	72	168	215	75	38	5.7	0	0	4	3.5	45
14	36	71	188	246	182	40	6.2	3	1	5	3.5	39
15	37	74	178	169	72	78	2.2	0	0	5	3.5	43
16	34	69	150	214	93	32	6.7	0	0	4	0	0
17	34	68	142	146	104	36	4.1	3	0	3	0	0
18	38	75	184	160	49	48	3.3	3	0	5	1.5	21
19	40	72	205	190	54	44	4.3	3	0	2	1.5	24
20	34	68	153	246	100	54	4.6	0	0	5	1.5	30
21	40	73	180	185	100	58	3.2	3	0		1.5	32
22	37	76	192	215	112	36	6.0	0	0	4	1.5	14
23	39	73	187	204	148	42	4.9	0	0		3.5	32
24	34	70	150	147	62	44	3.3	0	0	4	0	3
25	37	66	143	183	76	58	3.2	3	0		1.5	30
26	38	70	190	217	97	38	5.7	0	0	3	0	0
27	40	60	148	174	77	38	4.6	3	0	3	0	0
28	38	66	150	185	99	50	3.7	0	0	4	3.5	47
29	34	68	150	156	56	50	3.1	11	0	4	0	13
30	36	69	169	158	45	42	3.8	3	0	5	3.5	40
31	35	72	180	221	116	46	4.8	3	0	4	5.5	66
32	36	72	185	162	57	50	3.2	3	0	3	3.5	26
33	43	70	165	121	62	40	3.0	0	0	5	1.5	11
34	38	69	166	213	93	46	4.6	0	0	4	1.5	20
35	35	71	190	199	56	54	3.7	0	0		3.5	66
36	33	74	195	169	66	50	3.4	3	0	4	3.5	25
37	35	70	150	154	77	56	2.8	3	0	5	3.5	52
38	33	66	140	189	80	42	4.5	3	0	5	1.5	25
39	35	70	192	203	67	38	5.3	3	5		5.5	58
40	34	73	202	150	95	54	2.8	0	0		1.5	39

(continued on next page)

Table I-1. Raw Data from Test # 1

Appendix I

#	AGE	HT	WT	CHOL	TRIG	HDL	RATIO	ALCO	CIG	FIT	# SES	PTS
41	40	72	190	171	67	70	2.4	15	0	5	5.5	106
42	38	70	180	220	155	42	5.2	0	0		0	10
43	36	71	176	192	95	50	3.8	7	0	5	5.5	120
44	33	71	170	203	90	66	3.1	11	0	5	5.5	160
45	33	72	195	213	75	36	5.9	3	0	4	3.5	30
46	34	71	191	183	45	56	3.3	3	0		3.5	60
47	35	68	157	193	146	54	3.6	0	0	5	3.5	60
48	39	69	175	240	86	44	5.5	11	0		5.5	34
49	34	71	176	140	78	56	2.5	0	0	5	3.5	33
50	34	71	190	161	66	46	3.5	7	0	4	3.5	33
51	37	74	183	219	131	40	5.5	3	0	4	0	9
52	35	68	155	222	89	32	6.9	0	0	3	3.5	12
53	36	67	150	221	115	36	6.1	3	0	3	0	10
54	38	69	170	263	72	32	8.2	7	0	5	0	0
55	39	77	220	185	92	56	3.3	3	0		3.5	50
56	34	70	150	190	75	54	3.5	0	0	5	3.5	50
57	34	67	151	212	112	38	5.6	3	0	5	3.5	45
58	32	66	139	236	237	40	5.9	3	0	3	0	12
59	35	68	175	161	83	40	4.0	0	0		0	0
60	35	73	150	140	42	54	2.6	7	0	4	3.5	37
61	35	86	140	185	61	34	5.4	0	0	3	0	0
62	33	72	198	207	146	44	4.7	3	0	5	3.5	39
63	33	73	168	200	66	48	4.2	0	0	5	3.5	58
64	35	71	185	182	113	38	4.8	7	0	4	1.5	5
65	33	71	195	137	78	50	2.7	3	0	4	1.5	25
66	38	68	180	259	233	38	6.8	15	25	3	1.5	7
67	34	69	145	142	104	56	2.5	3	0	4	3.5	44
MEAN	35.8	70.5	173.7	189.0	98.7	46.7	4.3	3.5	1.1	4.2	2.6	34.9
SD	2.3	3.3	20.5	31.9	42.4	9.9	1.3	4.2	4.6	0.8	1.7	28.8

Table I-1. (continued)

Appendix I

#	AGE	HT	WT	CHOL	TRIG	HDL	RATIO	ALCO	CIG	FIT	# SES	PTS
1	35	70	187	195	159	46	4.2	11	0	4	5.5	50
2	36	69	172	187	129	36	5.2	15	0		3.5	44
3	35	73	224	221	150	46	4.8	0	15	3	3.5	30
4	34	72	195	129	121	38	3.4	0	0	4	3.5	46
5	35	71	198	197	101	48	4.1	11	15	4	5.5	36
6	36	69	167	171	58	44	3.9	11	0	4	5.5	130
7	32	71	145	139	152	38	3.7	3	0	5	3.5	32
8	34	70	175	198	88	36	5.5	7	0	4	5.5	35
9	37	73	202	159	73	58	2.7	0	0		3.5	56
10	39	69	170	200	137	54	3.7	0	0	3	3.5	34
11	36	66	160	181	73	42	4.3	0	0	3	3.5	34
12	37	71	195	236	159	52	4.5	7	0	5	3.5	86
13	40	72	168	232	103	62	3.7	0	0	4	3.5	39
14	36	71	188	236	189	42	5.6	3	0	5	3.5	63
15	37	74	178	155	68	44	3.5	0	0	5	5.5	71
16	35	69	150	194	99	56	3.5	3	0	4	3.5	32
17	35	68	142	173	170	54	3.2	3	0	4	5.5	36
18	38	75	184	194	95	36	5.4	7	0	5	0	19
19	41	72	205	196	99	48	4.1	3	0	3	5.5	30
20	34	68	153	235	108	46	5.1	0	0	5	3.5	53
21	40	73	186	204	91	42	4.9	3	0	3	3.5	32
22	37	76	192	194	107	46	4.2	3	0	5	3.5	53
23	40	73	192	216	223	46	4.7	0	0	5	3.5	48
24	34	70	150	155	61	50	3.1	0	0	4	0	30
25	37	66	143	177	62	58	3.1	3	0	4	3.5	29
26	39	70	190	213	85	42	5.1	3	0	4	3.5	32
27	40	69	148	185	73	40	4.6	3	0	4	3.5	26
28	39	66	150	195	106	42	4.6	0	0	4	3.5	58
29	35	68	150	160	92	38	4.2	15	0	5	3.5	36
30	37	69	169	171	62	44	3.9	3	0	5	3.5	68
31	35	72	174	201	113	48	4.2	3	0	4	5.5	88
32	36	73	185	157	92	58	2.7	3	0	3	5.5	37
33	43	70	165	142	60	44	3.2	3	0	5	3.5	36
34	38	69	166	209	106	48	4.4	0	0	5	5.5	80
35	36	71	190	202	65	50	4.0	0	0	5	5.5	120
36	33	74	190	190	65	56	3.4	3	0	3	1.5	30
37	36	70	150	137	79	50	2.7	3	0	5	5.5	44
38	33	66	140	206	93	52	4.0	3	0	5	3.5	110
39	36	71	192	171	102	54	3.2	3	5	4	3.5	63
40	35	73	202	164	111	38	4.3	0	0	3	3.5	33
41	40	72	185	152	58	38	4.0	15	0	5	5.5	104

(continued on next page)

Table I-2. Raw Data from Test # 2

Appendix I

#	AGE	HT	WT	CHOL	TRIG	HDL	RATIO	ALCO	CIG	FIT	#	SES PTS
42	38	70	175	175	95	44	4.0	0	0	4	5.5	30
43	36	71	176	181	68	44	4.1	11	0	5	3.5	108
44	34	71	170	200	76	44	4.5	11	0	5	5.5	162
45	34	72	195	210	87	46	4.6	0	0	4	1.5	35
46	34	71	191	180	49	44	4.1	7	0		5.5	56
47	36	68	157	188	126	48	3.9	0	0	5	3.5	59
48	39	69	175	230	64	44	5.2	7	0	4	5.5	39
49	35	71	183	131	141	38	3.4	0	0	4	3.5	33
50	34	71	190	183	77	36	5.1	7	0	3	3.5	30
51	37	74	183	177	141	48	3.7	3	0	4	3.5	32
52	35	68	155	229	147	48	4.8	0	0	3	3.5	30
53	36	67	150	219	118	46	4.8	3	0	3	1.5	11
54	38	69	158	231	83	42	5.5	3	0	5	3.5	57
55	39	77	220	182	142	42	4.3	3	0		5.5	30
56	34	70	150	196	76	50	3.9	0	0	5	5.5	68
57	34	67	151	237	155	38	6.2	3	0	5	3.5	62
58	32	66	145	253	201	36	7.0	3	0	4	3.5	32
59	36	69	175	139	100	36	3.9	0	0	3	1.5	19
60	35	73	150	147	51	38	3.9	7	0	4	3.5	42
61	35	68	135	153	55	40	3.8	0	0	4	5.5	28
62	39	72	198	202	116	54	3.7	3	0	5	5.5	78
63	33	73	168	185	80	40	4.6	0	0	5	3.5	44
64	35	71	185	215	136	70	3.1	7	0	5	3.5	47
65	34	71	195	129	91	44	2.9	3	0	4	3.5	29
66	38	68	180	259	194	52	5.0	15	25		5.5	18
67	35	69	145	166	79	40	4.2	3	0	5	3.5	47
MEAN	36.2	70.4	173.5	188.4	104.3	45.7	4.2	3.8	0.9	4.2	3.9	49.7
SD	2.3	2.4	20.6	31.0	39.2	7.0	0.8	4.2	4.0	0.7	1.3	28.5

Table I-2. (continued)

Appendix I

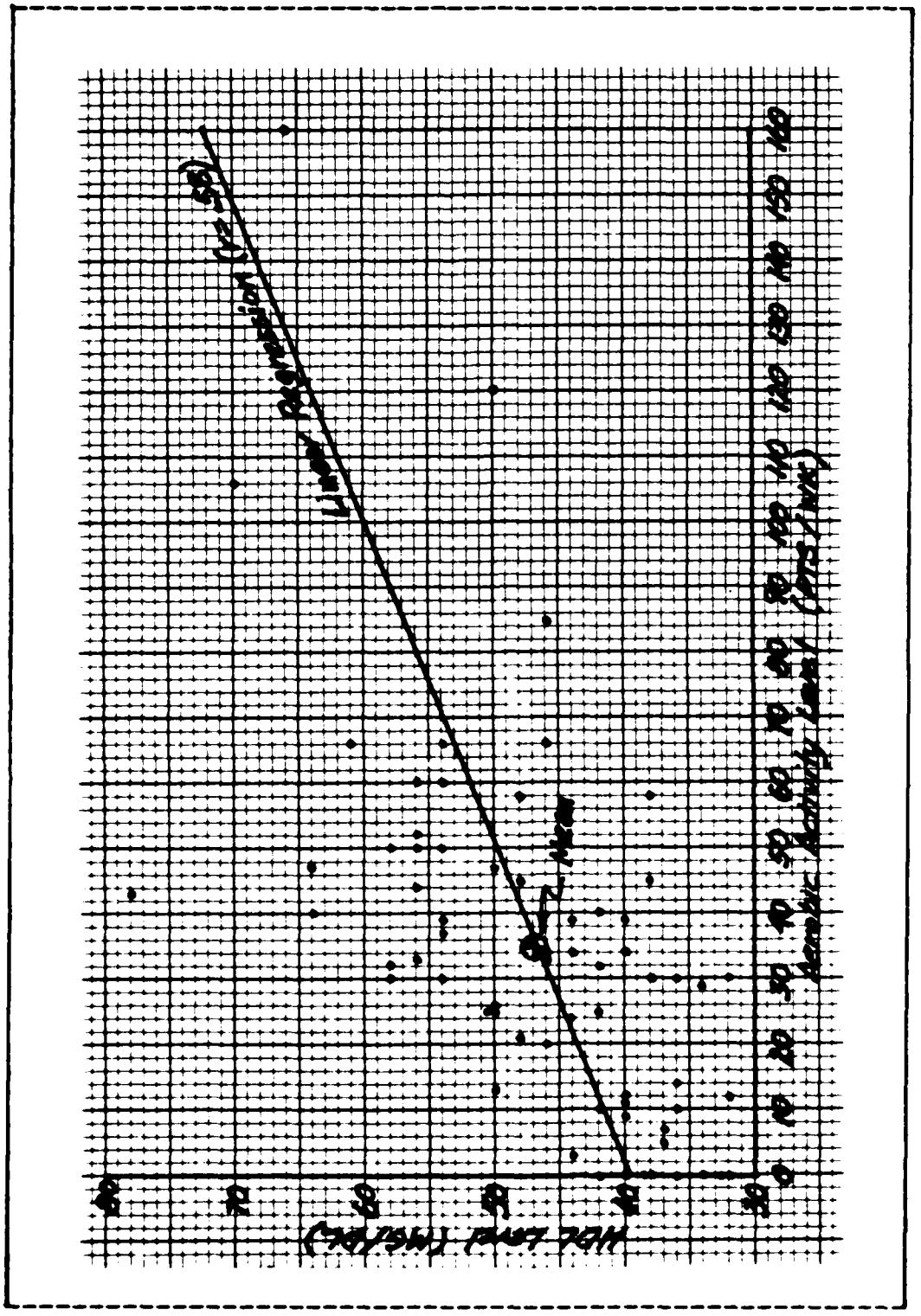

Figure I-1. HDL Level Versus Aerobic Activity Level--Test # 1

Appendix I

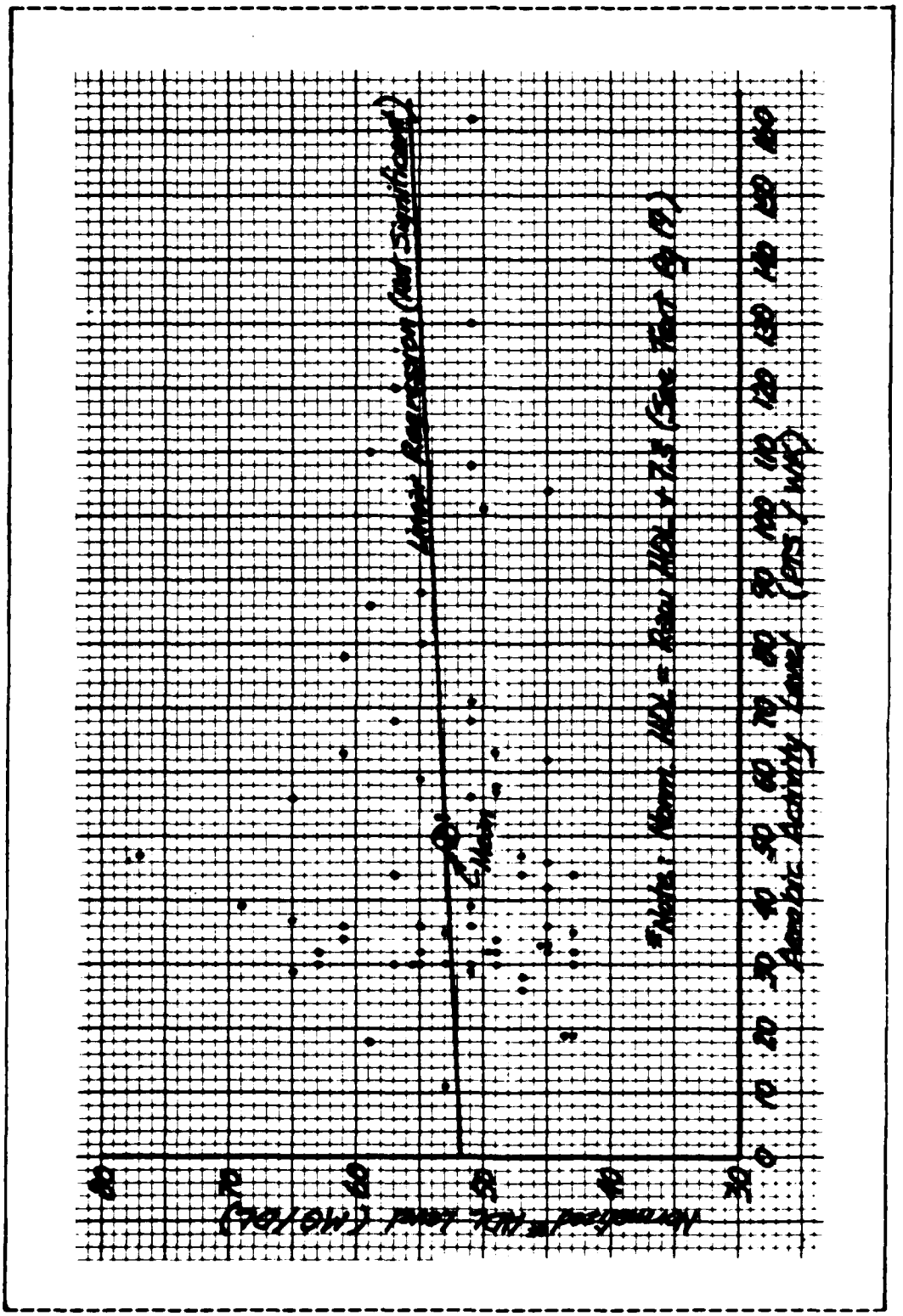

Figure I-2. HDL Level Versus Aerobic Activity Level--Test # 2

Appendix I

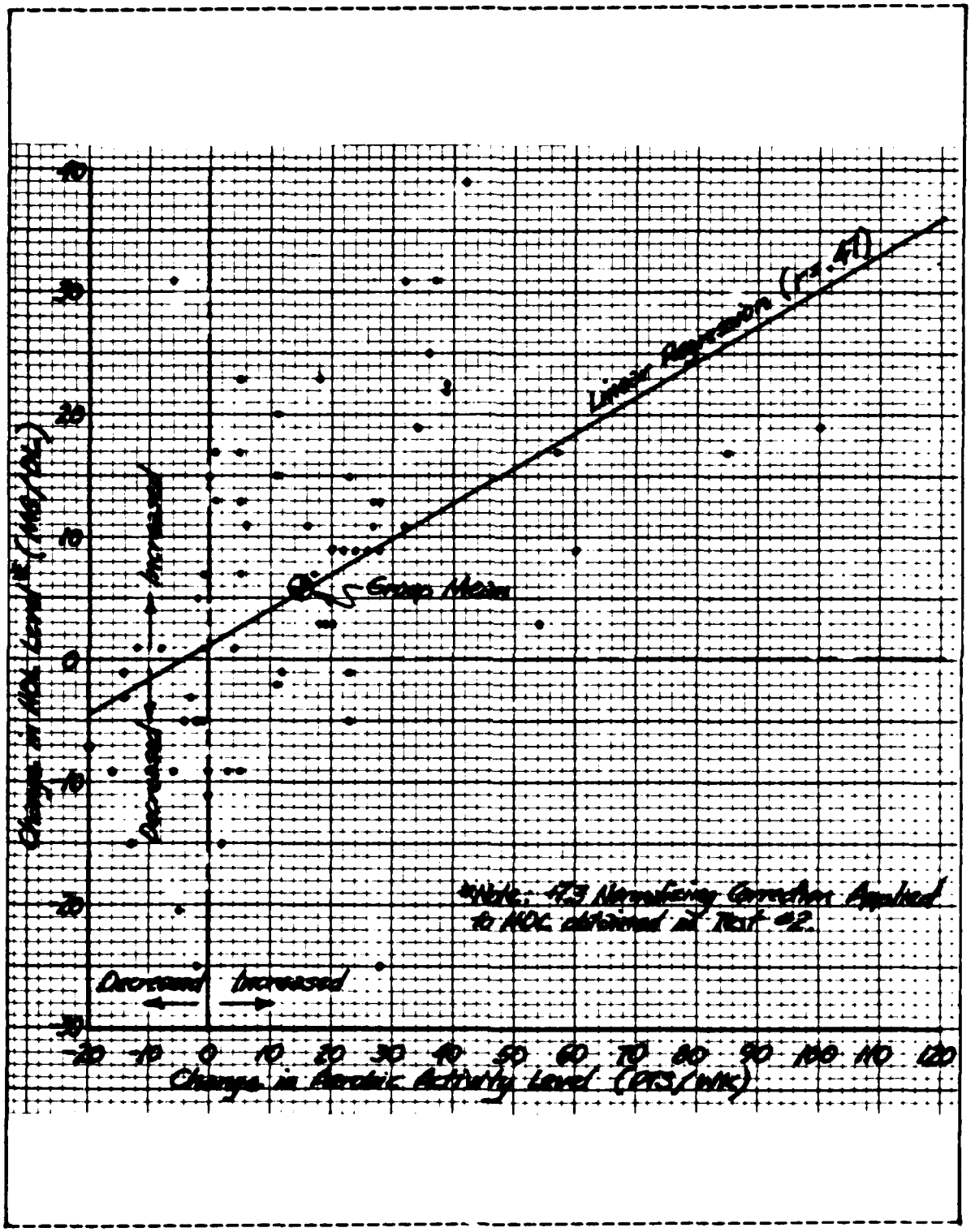

Figure I-3. Change in HDL Level Versus Change in Aerobic Activity Level

END

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